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Treatise on Surgical Infections

Frank Lamont Meleney, M.D.

ASSOCIATE PROFESSOR OF CLINICAL SURGERY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY

ASSOCIATE VISITING SURGEON
PRESBYTERIAN HOSPITAL
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Preface

THIS BOOK has been in the process of preparation for the last twenty years. During the first five-year period, data were being accumulated in the clinic and the laboratory with reference to the surgical infections appearing in the wards of the Presbyterian Hospital and to similar lesions of unusual interest in neighboring hospitals, which had been called to our attention.

Over a period of years, the infections developing in clean operative wounds served as a basis for an understanding of the various factors that play a role in sterile operating-room technic, and emphasized the importance of a continuous study of these infections to keep the surgeons 'bacteriologically minded' and to impress upon them the importance of maintaining the highest standards of 'sterile technic,' and their individual responsibility for it.

Routine aerobic and anaerobic cultures in all surgical infections, particularly in all chronic infections and 'infection problems,' revealed how often anaerobic organisms play a role in those diseases. This has led to an accumulation of interesting data that seem worthy of recording.

In 1935, the outline of this book was formulated and many chapters were completed, but just then the field of chemotherapy began to expand in a surprising manner, and it was soon apparent that the whole aspect of surgical infections might be greatly modified by the application of the new agents that had been discovered. Therefore, it was thought necessary to await their appraisal and to gain new experiences in their use in the treatment of all types of surgical infections.

Then came the ominous thunderings of war, the call for doctors, and the dispersion of laboratory workers, the anticipation of infection in war casualities, and the urgent need for an evaluation of old and new ways and means of preventing or combating these infections. It has taken a number of years to understand the limitations of these new chemotherapeutic agents. The brilliant results that were predicted for surgical lesions were often lacking as the indications for and limitations of these drugs became more clearly defined.

During the last five-year period, another new field of antibacterial therapy has been opened up, namely, that of antibiotics. With the experience gained with the sulfonamides, the study of the antibiotics has been carried out with more conservatism and greater understanding. This new field is very promising and, although its exploration has just begun, its fundamental principles have been established and it now

seems reasonable to present what knowledge we have. *Kaleidoscopic* changes, however, are to be expected in the near future, and possibilities increase by geometrical progression if we contemplate the synergism of the combined forces of chemotherapy and antibiotic therapy.

Before the time of Pasteur and Lister, surgical infections, particularly in hospital wards, ran their course in a characteristic fashion that was well recognized and frequently described by the medical authors of that period. We can be fairly certain now that those surgical lesions were for the most part due to mixed infections in which both the synergism and antagonism of bacteria played an important role.

During the subsequent period, following Lister and Pasteur, when it was understood that these infections were caused by bacteria and that they could be destroyed by appropriate means, the lesions more frequently developed from pure cultures of organisms. These infections also ran their characteristic course, which was well recognized by experienced surgeons. During the last fifty or sixty years, we have come to recognize the natural course of these diseases, both with and without the aid of surgery.

During this time, the physiological disturbances caused by infections, as well as the physical conditions favoring the development of infections, have gradually been clarified by various researches. During the last ten years, the course of surgical infections has been greatly altered, so that surgeons have had to build up an entirely new set of experiences regarding the course of these disorders, so modified by the chemotherapeutic and antibiotic agents.

Recently we have come to appreciate the fact that many resistant strains of pathogenic bacterial species, usually susceptible, are cropping up in the human community. These are doubtless the progeny of organisms that have come in contact with these new chemicals but that have been able to survive. Infections caused by these organisms may have certain features that are different from the original course of infections produced by these species and distinct also from the course they assumed when first treated with the specific antibacterial agent.

Just as surgeons in the Listerian era during the last sixty years had never seen the alarming infections that developed in surgical practice before the time of Lister, the coming generation of surgeons may never see infections following a course to which a surgeon of ten years ago was accustomed. This all clearly indicates that the study of surgical infections is not static but dynamic, constantly changing, always interesting, and often surprising.

Little by little, surgical infections have come under control: first, operating-room technics immediately broadened the scope of surgery; secondly, during the past decade, the hemolytic streptococcus, which

had for years baffled every bacteriologist who tried to study it, has lost its terror; thirdly, and very recently, the hemolytic staphylococci are seen yielding to penicillin. But there are still many gaps to close; chief among them is the problem of mixed infections, which figure so prominently in war wounds, compound fractures, and burns.

The goal of the surgical bacteriologist is that future state of affairs in which it will be possible to prevent bacteria from gaining a foothold in the human body, and if they have entered and established themselves, when it will be possible to stop their activity and bring about their death or elimination. This goal is not yet in sight. The struggle is still going on, and it is hoped that this book will aid in that venture. In short, the purpose of this book is threefold: (1) To maintain high standards of sterile technic. Therefore, certain sections of this book are particularly intended to help the supervisors of operating-room procedures. (2) To elucidate the bacteriological problems of surgery. This requires very careful laboratory work, co-ordinating the science of bacteriology with the art of surgery, enlarging our knowledge of anaerobic, as well as aerobic, technics, and ascertaining the cultural characteristics and growth requirements of organisms producing surgical infections. This book is particularly designed to bring the laboratory close to the clinic, and on that basis methods suggested by the results of research in the laboratory have been applied freely to the problems of infection in the surgical wards. Surgery is a mechanical aid to the body in its defense against infection. The surgeon must therefore be interested in and cognizant of the biological defenses that the body mobilizes against the invasion of micro-organisms. These have been briefly outlined. (3) To describe the natural course of surgical infections with and without surgical procedures and their therapeutic aids. For that reason, illustrative cases in various types of surgical infections have been briefly abstracted, bringing out certain pertinent facts that exemplify and illuminate general principles. Furthermore, the general principles of special kinds of therapy have been presented.

It is therefore with some trepidation, but with abundant hope, that I offer this book to hospital operating-room supervisors, bacteriological laboratory workers, and practicing surgeons, with the desire that it may help them in their labors and bring them a greater measure of success.

In this book, I have tried to correlate and co-ordinate all of my experiences with surgical infections during the last twenty-five years. These have been shared and in large measure amplified and clarified by my co-workers on the staffs of the Peking Union Medical College in China and the Columbia-Presbyterian Medical Center in New York. Grateful acknowledgment is made particularly to my laboratory associates, Miss Balbina Johnson, Dr. Helen Jern, Dr. Harold Harvey, Dr. Cornelius

Kraissl, Dr. John Lockwood, Dr. Edward Howes, Dr. Champ Lyons, Dr. Edwin Pulaski, and Dr. Alfred Longacre. Miss Johnson has written the final form of the chapters on laboratory techniques and the 'surgical' bacteria, Dr. Pulaski the chapter on antisepsis, and Dr. Kraissl the section on air sterilization. For their aid, I am very grateful.

The experiences of this group cover three periods of time, which may be designated as (1) before chemotherapy, (2) during chemotherapy, and (3) during antibiotic therapy. What the future holds no one knows, but we hope that steady progress will be made toward the goal we have set.

My gratitude is immeasurable to my two chiefs, Dr. Adrian S. Taylor in Peking and Dr. Allen O. Whipple in New York, both of whom invariably gave me the most loyal support, freedom of action, and encouragement in my endeavors. To Dr. Hans Zinsser I owe my fundamental training in bacteriology. To my confreres in the surgical department who have so generously let me use their case records, I give thanks. To my sister Grace and to my secretaries, Miss Lydia Titus and Miss Elizabeth Clark, I am grateful for their aid with bibliographies, indices, and proofreading. Finally, to my wife, without whose aid, encouragement, and patient waiting this book could never have been written, I extend my apologies, as well as my gratitude and love.

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F. L. M.

New York City
March 1948

The development of modern medicine toward increasing precision of diagnosis and therapy is, to a large extent, due to well-organized cooperation of laboratory and clinic. Today there is a continuous flow of principles and methods from the fundamental sciences into the applied science of human biology. And the channels that direct this flow are the so-called preclinical laboratories of medical schools. These laboratories thus fulfil the function of carrying the newer knowledge of chemistry, physics, and biology into medicine and of transmitting it, in utilizable form, to the clinical investigator. It is the obligation of meeting these responsibilities that has converted such formerly purely medical laboratories into organizations in which fundamentally trained scholars can collaborate with colleagues of more specific medical orientation.

This movement of drawing the basic sciences into the service of medicine is not a new one. It began with Lavoisier, Gay-Lussac, and others, who applied quantitative chemical methods to physiology, and it became inevitable with Pasteur, Claude Bernard, Liebig, Ehrlich, and many others, who demonstrated that medical progress must take much deeper scientific root than mere bedside and mortuary study. But even when—after considerable lag—this principle had become practically applied, in itself it was not enough. It was necessary to arrange further forward bridgeheads between the preclinical laboratories and the clinics themselves. This has been a more recent accomplishment and was not firmly achieved until—a generation ago—it became the custom to appoint to clinical chairs men of laboratory training and talent for research. The result was the establishment, within clinics, of special laboratories that tie up with departments of physiology, pathology, et cetera, as the latter, in their turn, draw upon chemistry, physics, and their branches.

It would seem, therefore, that a satisfactory system has been set up whereby medical scholarship can be constantly transfused with the advances of all applicable scientific discoveries. And to some extent this is true. But the velocity of discovery is increasing so vigorously that this system requires constant readjustment as new and highly specialized techniques become available for medical purposes. It is necessary to exercise continuous vigilance to prevent individual subdivisions, originally intended as links between one discipline and another—all of them together pointing toward clinical medicine—from succumbing to the automatic tendency of becoming hermetically sealed within the confines of their own particular problems and interests. One of the answers to this

difficulty lies in the development of scholars who, both in training and in actual service, form links between the several divisions.

As far as infectious diseases are concerned, such liaison is being accomplished by a group of amphibians who, like Dr. Meleney, though serving in the clinics, return from time to time to the bacteriological laboratory. At Harvard it has become a well-established custom for young physicians and surgeons who have completed their hospital services to return for one or two years of intensive study and research to the bacteriological department, not with the intention of becoming bacteriologists but rather of preparing themselves for work with patients. Such men often retain their official connections with the preclinical laboratory and prove invaluable in guiding medical students in the application of modern bacteriological and immunological methods to the problems of the bedside.

In the initiation of such a scheme, Dr. Meleney has been a pioneer. It was largely the success of his co-ordinating labors that encouraged our own confidence in its soundness. And the present book—the result of his experience—will prove of great value in stimulating others to follow where he has led.

July 1940

[Dr. Zinsser read the first draft of the manuscript and wrote this foreword shortly before his death.]

Introduction by Allen O. Whipple

DR. ZINSSER'S foreword brings back memories of the days in his laboratory at the College of Physicians and Surgeons. In his inimitable way he was giving an informal course in the bacteriology of surgical infections to a group of six surgeons from the Presbyterian Hospital. The youngest of these was Frank L. Meleney, then an instructor. He is the only one of that group who has followed seriously and intensively the study of the etiology and prevention of the infectious lesions most commonly encountered in surgery. This volume is convincing proof of the sterling quality of his persistent interest and accomplishment in this field.

It is fair to say that since Lister's time no surgeon has studied so intensively and has made so many original contributions to the subject of the bacteriology of infection and inflammation requiring surgical therapy as has Dr. Meleney in the past twenty-five years. A review of the titles of the chapters of this book reveals the comprehensive scope, not only of the bacteriology, but also of the prevention and treatment of surgical infections. And the interesting fact is that Dr. Meleney bases these chapters on original observations corroborated by a long and rich experience.

This experience has not been gained without trial and tribulation. At times his studies and conclusions have run counter to complacent tradition and to wishful thinking. An example of the former was his convincing proof, in 1926, that infections in clean wounds at the Presbyterian Hospital and other university clinics were more common than was generally believed and that careful masking of the nose was as important as masking of the mouth as a means of preventing the serious hemolytic streptococcus infections. An example of the latter was the disappointing but convincing evidence that the sulfonamides applied locally to contaminated wounds in civilians did not prevent the infections that premature and uncontrolled reports had heralded to an eagerly receptive profession. Since then the experience of surgeons in combat areas in various parts of the world have corroborated his report from the Sub-Committee on Infected Wounds and Burns of the National Research Council.

This comprehensive text comes at a most opportune time when the attention of the surgical profession is focused on the potentialities of chemotherapeutic and antibiotic agents. Dr. Meleney's original contribution to the etiology and treatment of anaerobic infections, and to the development of antibiotic agents in the control of Gram-negative and

mixed organisms, make these new chapters in surgical therapy of ou
standing importance.

Another, and his most constructive accomplishment, is the interest l
has created in the study of the bacteriology of surgical infections in ma
of the surgical clinics of this and other countries. Surgeons like Harve
Lyons, Lockwood, Longacre, Sandusky, and Pulaski started their wo
in surgical bacteriology in Dr. Meleney's laboratory, and have demo
strated, as he has for many years, the value of such laboratories
civilian and military hospitals.

Only those who have worked with him can understand the time ar
effort that he has given to these bacteriological studies in addition to h
active clinical surgery, and only those of his friends and associates wh
have tried to beat him at golf or at chess can appreciate his quality
refusing to acknowledge the possibility of failure or defeat. That quali
is one of the reasons for this book.

It gives me, his admiring associate for some twenty-five years, gre
pleasure to contribute this foreword to a great work.

April 1946

Contents

TREATISE ON SURGICAL INFECTIONS

Historical

I. THE ORIGIN OF BACTERIAL INFECTION IN THE HUMAN BODY

LONG BEFORE man walked the earth, bacteria lived and grew and propagated their kind. Osborn[1] believed that bacteria were primordial forms of life and that their parasitic characteristics were secondarily acquired. He said, 'It is probable that bacteria-like organisms prepared both the earth and the ocean for the further evolution of plants and animals and that life passed through a very long bacterial stage.'

The invasion of dead tissues by bacteria is one of the most important processes of nature, for by it these tissues are broken down into those elemental substances that in the cycle of life can again be taken up and synthesized into plant form. Plants, in turn, are converted by digestion into animal tissues. A bacterialess earth and a bacterialess ocean would soon be uninhabitable both for plants or for animals. It may well be that *in the beginning of human infection, bacteria invaded gangrenous or* dead tissue, still attached to a living body, with the unexpected result that instead of reducing it to its elements, they met the defense of the living body, to which they had to adapt themselves or be destroyed. Then followed the modification of the species of bacteria, either by the development of qualities that would enable them to survive, or by the survival of those progeny able to live within the living tissue. Thus, in time, new bacterial species evolved that could live only within certain living tissues. Then, in order that such species might persist, it became essential that they should be transferred directly from one living host to a similar living host.

Most of this adaptation occurred long before recorded human history, and we can only assume the gradual steps by which this change took place during the eons of time, compared with which the 5000 years of recorded history are but a short span. Even during this relatively brief period, however, further evolution in the interrelationship between bacterial species and the human race has probably been going on. Certain it is that for almost all this length of time, man has been entirely unaware

of the processes of nature by which he fell a victim either to the degenerative changes leading to his slow death or to the destructive action of bacteria leading to his sudden death. Nor was man able to discover how these processes might be interrupted so as to preserve or prolong life. As man discovers the laws governing such processes, it is probable that he can control and direct them so as to eliminate the unfavorable and preserve the favorable aspects of evolution. In his *History of Medicine, Surgery and Anatomy*, Hamilton[2] writes:

It might have been expected that the origin of a branch of knowledge so conducive to the welfare and so essential to the preservation of the human race as the Art of Healing, would have been preserved among men with a kind of religious veneration and been traceable to its remotest source with a distinction proportionate to its interest and a precision equal to its importance.

But such is not the case.

II. THE MOST ANCIENT RECORDS

Although it is outside the range of this book to delve extensively into the ancient history of medicine, it is of interest to call to mind some of the landmarks of medicine where they apply to surgical infections. Hippocrates is generally believed to be the Father of Medicine, but it should not be forgotten that Egypt had a well-developed civilization long before his day, and records have come down to us through excavations indicating that the art of medicine had reached a high stage of development in those early days of recorded history. Inscriptions on stone and papyrus all point to Imhotep (*c.*3000 B.C.) as the founder of Egyptian medicine, and, although none of his own writings is available, references to him indicate his extensive experience with the sick, in both the diagnosis and the treatment of disease. The chief medical records of the ancient Egyptians that have come down to us are the Ebers and the Edwin Smith papyri, which were found entombed in Thebes eighty-five years ago. Their contents had to await interpreters, and even now there are many terms that are not understandable. The Ebers papyrus, written *c.*1550 B.C., was evidently compiled from a series of at least forty books written many centuries earlier. It includes detailed anatomic studies; long lists of prescriptions for numerous ailments, with doses and method of administration; the surgical treatment of fractures, boils, tumors, and suppurating sores, with directions for the treatment of wounds.[3] From symptoms described in this papyrus, 250 different diseases may be recognized, and this record together with clear evidence in the human bodies, so well preserved by the fine Egyptian art of embalming, gave ample proof that spinal tuberculosis, gallstones, appendicitis, gout, rheumatoid

arthritis, and dental and mastoid caries existed in those ancient days.[4] The Edwin Smith papyrus, brought to light about the same time, though not from the same tomb, is a somewhat older document, probably written a century earlier. It gives detailed descriptions of a number of individual cases—chiefly wounds of the head, face, neck, and spine. It was evidently the scribe's intent to cover systematically all parts of the body, but something interrupted him and he stopped in the middle of a sentence while describing a case of spinal disease. We find scant reference to any signs of inflammation or infection in the wounds he described, but he gave us just enough to know that infections, as we know them now, did occur.[5]

There is a long gap of 1000 years between these records and Hippocrates, a gap shrouded in mystery. The records of these years are few, and bear almost no testimony regarding the medical practice of that millennium. Herodotus affirms that

> The Assyrians even at the time of the greatest splendor and power of the Babylonian Empire, 900-550 B.C., had no physicians, but were in the habit of exposing the sick in the market place that they might confer about their disease with the passing multitude. If the passers-by had themselves been afflicted with the same disease as the sick person or had seen others so afflicted, they advised him to have recourse to the same treatment as that by which they escaped or as they had known to cure others.[6]

Infections that often develop in accidental wounds are of course of serious consequence in times of armed conflict, so incessant among the ancients, and yet few records of them have come down to us. This lack is probably due to two principal causes. First, it is well established by the writings of the Hebrews, of Homer, Herodotus, and many others that notwithstanding the immense armies of the ancients and the immense slaughter that took place in their battles, the dead being numbered by tens and even hundreds of thousands, few or no wounded survived the immediate conflict. The contending armies came to close quarters and engaged in desperate hand-to-hand fights, always continuing until one or the other antagonist was slain—few or no wounded survived, and the prisoners who were captured were either immediately destroyed if wounded, or sold as slaves if unscathed. Second, physicians who were present at the battles and sieges of those times appear to have served not in an official capacity as army surgeons but as soldiers.

III. THE AGE OF HIPPOCRATES

It is not until we come to the age of Hippocrates at 500 B.C. that we have any volume of medical literature.

Many records attributed to Hippocrates are probably not authentic, and doubtless many actually made by him were lost, but we gather from the material available that Hippocrates' major contribution to medical progress was the result of his careful observations and his systematic study of disease.

Hippocrates wrote on many different medical subjects. He described in detail a number of cases of obvious infection and noted their onset, their course, and their seasonal incidence. He noted the importance of contagion, but credited the spread of the disease largely to winds and other air currents. He called such cases 'epidemics,' believing that they were afflictions visited 'upon the people,' a meaning quite different from the modern usage of the term. For example, he includes a number of cases that are obviously puerperal fever. One was an evident cellulitis with the pathognomonic sign of hemolytic streptococcus gangrene. I do not believe that this was gas gangrene as suggested by Millar,[7] or erysipelas as believed by Adams.[8] The infection was probably due to a trivial wound around the nail. Hippocrates' description is worth repeating:

Criton, in Thasus, while still on foot and going about, was seized with a violent pain in the great toe; he took to bed the same day had rigors and nausea, recovered his heat slightly, at night was delirious. On the second, swelling of the whole foot, and about the ankle erythema with distention and small bullae [phlyctenae], acute fever; he became furiously deranged, discharges bilious, unmixed, and rather frequent. He died on the second day from the commencement.[9]

Hippocrates described ulcers very similar to those seen today in which bacteria play an important role. He said, 'When the parts adjoining an ulcer are inflamed, the ulcer is not disposed to heal until the inflammation subsides; nor when the surrounding parts are blackened by mortification is the ulcer disposed to heal unless you bring the surrounding parts into a healthy condition.'[10] Of corroding ulcers, he said: 'Those which are phagedenic, spread and corrode most powerfully. In this case the parts surrounding the sore will have a black or sublivid appearance.'[11] These are obviously descriptions of infection, which, though they occurred 2400 years ago, are similar to those existing today.

Little or nothing was added to medical knowledge in the next five centuries, and then Galen with his authoritative power closed the door to further progress for the next 1300 years. Galen's scanty references to surgical infections include the description of a 'complaint accompanied with carbuncles which afforded frequent opportunities for observing the disposition of the muscles in several places in which they had been exposed through the effects of the complaint.'[12]

Rhazes, the illustrious Arabian writer, lived in the ninth century. Although he stood out as a giant among the writers and observers of medical and surgical disorders and was the author of over two hundred books, he acknowledged his own debt to those who had gone before him, and uttered a profound truth when he said.

The accumulation of knowledge is gradual and progressive and perfect originality is one of those Utopian perfections which is only to be looked for among those writers who have the good fortune to enter upon untrodden ground. It is not the mere accumulation of isolated facts which constitute science but the digestion of those facts into a connected and intelligible system, and he is the greatest benefactor of the cause of learning who, taking up the works of his predecessors in any department of knowledge, illustrates them by his observations and enriches them by the incorporation of his own discoveries.[14]

The Arabian learning was later (c. A.D. 1000) driven westward through Egypt to Morocco and then over to Spain, where it centered in Cordova. A university was formed there that was noted for its library of 250,000 volumes. Avenzoar was the chief physician in this movement. He described many types of surgical infections, among them subdiaphragmatic abscess, from which he himself suffered. He also described abscess of the pericardium and adherent pericarditis.[15]

From A.D. 1000 to 1500, medicine was for the most part in the hands of a degenerate priesthood, who used it for monetary gain and, because of their ignorance of historical and traditional medicine, depended largely on charms and incantations. Gradually, however, in the Benedictine monastery, monks began to study seriously, and out of this study gradually emerged the School of Salerno. Salerno became the center from which the Crusaders departed and to which they returned from the Holy Land. Little is known of the surgical infections that undoubtedly occurred during those ceaseless wars, probably because of the distance of this medical center from the actual scene of conflict.

Other cities in Italy during this period began to gain a reputation as centers of medical teaching. Among these was Bologna, where Theodoric began a new principle in the treatment of wounds, the principle of meticulous cleanliness. For centuries before his time it had been the custom to leave wounds open and stimulate suppuration in them, but Theodoric emphasized the thorough cleaning and early closure of the wound. In 1266 he wrote a treatise on surgery in which he said:

It is not necessary as Roger and as Roland have written and as many of their disciples teach and all modern surgeons profess, that pus should be generated in wounds. No error can be greater than this. Such a practice is

indeed to hinder nature, to prolong the disease and to prevent the conglutination and consolidation of the wound.[15]

The field of surgical infections became enormously increased by the invention and use of firearms, which produced more extensively contused wounds with more damage to muscles and bones than had any of the former implements of war. The speed and momentum of the missiles enormously increased, and soil and clothing were carried into the tissues more deeply. The surgeons of that day were slow in coping with this new and difficult problem. They believed that these wounds were poisoned by the gunpowder, not realizing that it was the accompanying contamination that was doing the damage. They tried to neutralize the poison by the application of wines and oils of various kinds, which were never very effective. Guy de Chauliac advocated hot wine, and later surgeons, hot oils. They began to notice new and varied features in the development of infection in these wounds, and new terms such as 'corrosive' and 'putrefactive' began to appear in their writings. Wounds exhibiting such alarming aspects seemed to require more heroic methods of treatment, and thus came into use such drastic measures as boiling oil, strong acids, or the actual cautery. But in the second half of the sixteenth century came a period of reaction to these violent treatments.

In this new period Ambroise Paré stands out as the great surgeon, a man of wide human sympathy, modest concerning his own attainments, yet keen in observation, strong in self-confidence, and firm in will. He is deserving of our particular attention.

He followed the armies of France in victory and defeat. He observed the progress of war wounds and their subsequent infection, and gained an enviable reputation for his prognostic acumen and for his ingenuity in devising treatment. Yet time and again in explaining his success he stated simply, 'I dressed him, but God healed him.'

Certain quotations from his *Journeys in Diverse Places* will illustrate many, but not all of the kinds of surgical infections he saw, and will clearly indicate the virtues I have briefly mentioned.

Now I was at this time a fresh-water soldier; I had not yet seen wounds made by gunshot at the first dressing. It is true, I had read in John de Vigo, first book, *Of Wounds in General*, eighth chapter, that wounds made by firearms partake of venenosity, by reason of the powder; and for their cure he bids you cauterize them with oil of elders scalding hot, mixed with a little treacle. And to make no mistake, before I would use the said oil, knowing this was to bring great pain to the patient, I asked first before I applied it, what the other surgeons did for the first dressing; which was to put the said oil, boiling well, into the wounds, with tents and setons; wherefore I took courage to do as they did. At last my oil ran short, and I was forced instead thereof to apply a

digestive made of the yolks of eggs, oil of roses, and turpentine. In the night I could not sleep in quiet, fearing some default in not cauterizing, that I should find the wounded to whom I had not used the said oil dead from the poison of their wounds, which made me rise very early to visit them, where beyond my expectation I found that those to whom I had applied my digestive medicament had but little pain, and their wounds without inflammation or swelling, having rested fairly well that night; the others, to whom the boiling oil was used, I found feverish, with great pain and swelling about the edges of their wounds. Then I resolved never more to burn thus cruelly poor men with gunshot wounds.[14]

Of another occasion he wrote:

Their wounds were very putrid, and full of worms, with gangrene, and corruption; and I had to make free play with the knife to cut off what was corrupt, which was not done without amputation of arms and legs, and also sundry trepannings. And to correct and stop the corruption, and kill the worms in their wounds, I washed them with Aegyptiacum dissolved in wine and eau-de-vie, and did all I could for them, but in spite of all my care many of them died.[15]

His prize account reads as follows:

The King sent for me and bade me go and see M. d'Auret, and give him all the help I could, to heal him of his wound. I told him I would employ all the little knowledge it had pleased God to give me.

I found him in a high fever, his eyes deep sunken, with a moribund and yellowish face, his tongue dry and parched, and the whole body much wasted and lean, the voice low as of a man very near death, and I found his thigh much inflamed, suppurating, and ulcerated, discharging a greenish and very offensive sanies. I probed it with a silver probe, wherewith I found a large cavity in the middle of the thigh, and others around the knee, sanious and cuniculate: also several scales of bone, some loose, others not. The leg was greatly swelled, and imbued with a pituitous humor . . . and bent and drawn back. There was a large bedsore; he could rest neither day nor night, and had no appetite to eat, but very thirsty. I was told he often fell into faintness of the heart, and sometimes as in epilepsy; and often he felt sick, with such trembling he could not carry his hands to his mouth. . . It seemed to me there was little hope he would escape death. All the same, to give him courage and good hope, I told him I would soon set him on his legs, by the grace of God, and the help of his physicians and surgeons. . .

Having seen him, I went to walk in a garden, and prayed God he would show me this grace, that he should recover; and that He would bless our hands and our medicaments to fight such a complication of diseases.

After dinner, we began our consultation, all the physicians and surgeons together. . . I began to say to the surgeons that I was astonished they had not made incisions in M. le Marquis' thigh. . . They answered me: 'Never would he consent to it'; indeed, it was near two months since they had been

able to get leave to put clean sheets on his bed; and one scarce dared touch the coverlet, so great was his pain. Then I said, 'To heal him, we must touch something else than the coverlet of his bed' Each said what he thought of the malady of the patient, and in conclusion, they all held it hopeless. I told them there was still some hope, because he was young, and God and Nature sometimes do things which seem to physicians and surgeons impossible.

To restore the warmth and nourishment of the body, general frictions must be made with hot cloths, above, below, to right, to left, and around, to draw the blood and the vital spirits from within outward. . . For the bedsore, he must be put in a fresh, soft bed, with clean shirt and sheets. . . I said we must first ease the pain, making openings in the thigh to let out the matter. . . Secondly, having regard to the great swelling and coldness of the limb, we must apply hot bricks around it, and sprinkle them with a decoction of nerval herbs in wine and vinegar, and wrap them in napkins; and to his feet, an earthenware bottle filled with decoction, corked, and wrapped in cloths. Then the thigh, and the whole of the leg, must be fomented with a decoction made of sage, rosemary, thyme, lavender, flowers of chamomile and melilet, red roses boiled in white wine, with a drying powder made of oak-ashes and a little vinegar and a half a handful of salt. . . Thirdly, we must apply to the bedsore a large plaster made of the desiccative red ointment and of Unguentum Comitissoe, equal parts, mixed together, to ease his pain and dry the ulcer, and he must have a little pillow of down, to keep all pressure off it. . . And for the strengthening of his heart, we must apply over a refrigerant of oil of water-lilies, ointment of roses, and a little saffron, dissolved in rose-vinegar and treacle, spread on a piece of red cloth. For the syncope, from exhaustion of the natural forces, troubling the brain, he must have good nourishment full of juices. For the great pain in his head, his hair must be cut, and his head rubbed with rose-vinegar just warm, and a double cloth steeped in it and put there, also a forehead-cloth, of oil of roses and water-lilies and poppies, and a little opium and rose-vinegar, with a little camphor, and changed from time to time. Moreover, we must allow him to smell flowers of henbane and water-lilies, bruised with vinegar and rose-water, with a little camphor, all wrapped in a handkerchief, to be held some time to his nose. . . And we must make artificial rain, pouring water from some high place into a cauldron, that he may hear the sound of it; by which means sleep shall be provoked on him. As for the contraction of his leg, there is hope of righting it after we have let out the pus and other humors pent up in the thigh, and have rubbed the whole knee with ointment of mallows, and oil of lilies, and a little eau-de-vie, and wrapped it in black wool with the grease left in it; and if we put under the knee a feather pillow doubled, little by little we shall straighten the leg.

The consultation ended, we went back to the patient, and I made three openings in his thigh. . . Two or three hours later, I got a bed made near his old one, with fair white sheets on it; then a strong man put him in it, and he was thankful to be taken out of his foul stinking bed. Soon after, he asked to sleep, which he did for near four hours.

The following days, I made injections, into the depth and cavities of the

ulcers, of Aegyptiacum dissolved sometimes in eau-de-vie, other times
I applied compresses to the bottom of the sinous tracts, to cleanse and
soft spongy flesh, and hollow leaden tents, that the sanies might alw
a way out, and above them a large plaster of Diacalcitheos dissolved
And I bandaged him so skillfully that he had no pain, and when the
gone, the fever began at once to abate

Then, when I saw him beginning to be well, I told him we must h
and violins, and a buffoon to make him laugh, which he did. In one
we got him into a chair, and he had himself carried about in his ga
at the door of his château to see everybody passing by [18]

The example of Paré was gradually followed by those who ca
him—the more vigorous and destructive methods of wound tr
were gradually abandoned, and gentler handling and more
agents were employed. It was gradually discovered that certain
ments would prevent or check the processes of putrefaction. Su
stances were called 'antiseptics'

The term was first used by Place in 1721 in his treatise on the
He says:

As this phenomenon shows the motion of the pestilential poiso
putrefactive, it makes the use of antisepticks a reasonable way to o
Whatever resists and is preservative against putrefaction admits no
generation of insects . . . the same virtues that preserved dead bod
insects and putrefaction, I know no reason why they should not pres
same bodies living from the same thing [19]

One of the outstanding investigators in the early days of an
was Pringle, who was led to this study because of his extensive ex
with putrid distempers during his service with the British
Flanders in 1750.[20] Likewise, a French lady, Madame d'Arconvi
retired from society because of a facial disfigurement following s
at the age of twenty-three, devoted her life to a study of antisep
wrote a treatise of 600 pages on the subject of her researches
period of ten years.[21]

In 1770, the Academy of Sciences of Dijon offered a prize for
treatise on antiseptics. It was won by a Frenchman named Bor
who expressed the theory that putrefaction in a living body is ca
the separation from it of too great a quantity of air. He believed
chief use of an antiseptic ought to be to prevent that eleme
escaping or to replace it in the tissues.[22] This foreshadowed the
oxidizing agents in preventing putrefaction by anaerobic organis

Strangely enough, the study of the causes of putrefaction f
rather than preceded the study of its prevention. It was fou
heating, freezing, or hermetically sealing would prevent putre

but the cause of putrefaction baffled scientific investigation for decades after these facts were known. Finally, in 1835, Bassi began a study of *muscadine*—a disease of silkworms—and found it to be caused by a parasite that could be destroyed by appropriate means. On the basis of his findings, he predicted that contagious diseases, such as smallpox, gangrene, plague, et cetera, would be found to be caused by animal or vegetable parasites that likewise might be destroyed.[23]

Several decades passed before Pasteur began his studies on the fermentation of alcoholic beverages. He found that this was caused by micro-organisms that he could isolate and grow and destroy by heating.[24] It was but a step to prove that organisms were also responsible for putrefactive and disease processes.[25] He also proved that spontaneous generation was impossible and that the air, of itself, played no part in either fermentation or putrefaction, if the organisms it contained could be filtered from it.[26] This is the background of modern surgery.

Let us pause for a few minutes to consider the state of surgery at this time. To make it concrete and vivid in our minds, let us consider the question of surgical infections in the American Civil War as exemplified by the condition known as 'hospital gangrene.'

IV. HOSPITAL GANGRENE IN THE CIVIL WAR

Joseph Jones, a Confederate Army surgeon, became profoundly interested in the question of hospital gangrene, the *bête noire* of the wounded man and the army surgeons on both sides in the Civil War. After the war was over he was directed by the United States Sanitary Commission to review the records on both sides and report his findings. Records were scanty, but recollections were vivid in the minds of the surgeons who had had the responsibility for the care of those cases. Jones decided to tap that fund of knowledge by sending out a most elaborate questionnaire to the surgeons of both armies. Innumerable questions covering all aspects of the problem were asked and the answers, plus Jones's own personal experience and his review of the literature, form the basis of his truly extraordinary report.

By way of introduction, Jones wrote:

> From the facts available concerning the mode of warfare of the ancients, the imperfection of their medical knowledge, and of their arrangements for the treatment of the sick of their armies, and the comparatively modern origin of military and civil hospitals, we are justified in the assertion, that the mere absence of special descriptions of this disease in the writing of the ancients, is no proof that Hospital Gangrene is not a disease of great antiquity. The essential conditions for the origin and spread of this disease were frequently present in ancient times and probably occurred.[27]

Pouteau, a dresser in the Hôtel Dieu in Lyons, gave the first description of hospital gangrene and gave it its name. He was himself a victim of the disease and his report appeared in his posthumous publications in 1783[25] After that date the name was generally accepted and was applied to the common forms of destructive gangrenous lesions so prevalent in all hospitals at that period. Soon afterward, reports appeared in the medical literature of many European countries. In 1780 upward of 200 cases occurred in a Naval hospital in New York. In 1800 the disease broke out on an English vessel sailing from Martinique to Britain. 'Every little scratch or injury degenerated into a bad gangrenous ulcer.' 'Sixty bodies 'were thrown overboard by one vessel in thirty-six hours in her passage from the South of France to the Bosphorus.'[26] Gross reported that it was common in the hospitals of the Union Army during the Civil War in Annapolis, Washington, Baltimore, New York, Louisville, and Frederick. He said, 'If inquiry be made into the causes of this variety of gangrene it will hardly be found to reward our labor.'[26] Jones, however, undertook *the task and he has given us the most varied description of the disease,* as well as the most varied theories regarding its cause, after reviewing the literature as far back as Hippocrates and analyzing the questionnaires. His data will be condensed and presented in four parts:

1. Its occurrence in the Civil War
2. Theories as to its cause
3. The variety of its clinical aspects indicating that it was a heterogeneous group embracing all of the different types of acute and chronic infectious gangrene
4. Its treatment

Gangrene occurred chiefly in hospitals and temporary shelters where sick and wounded soldiers were crowded together with poor ventilation and insufficient supply of utensils, bandages, lint, and instruments. Often these soldiers were transported in large numbers crowded together in closed boxcars without any cleansing of their filthy wounds.

On one occasion a large number of wounded Federal prisoners were captured and were crowded into two churches. Hospital gangrene appeared a few days later and not a single wounded soldier escaped the disease. It was observed that those in the upper galleries were more severely afflicted than those on the ground floor, from whom 'the exhalations from the lungs and putrid wounds ascended.' In one southern prison, containing over 10,000 Federal soldiers, a third of those incarcerated, died within seven months. Diarrhea, dysentery, scurvy, and hospital gangrene were the main causes of this fearful mortality. Three hundred and eighty-five cases of hospital gangrene developed in one hospital in three months' time and a sixth of those afflicted died.[27]

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Concerning etiology, the observations of some seemed to indicate that it was primarily a general disorder with local manifestations. They noted first a constitutional weakness leading to an outbreak of the disease in some trivial wound such as a scratch or an insect bite or even without any apparent break in the skin. I quote:

In the progress of the constitutional symptoms, a general uneasiness is felt before any visible changes take place in the wound or sore which is attacked with hospital gangrene, the tongue becomes foul, with a sensation of bitterness in the mouth, and appetite decreases and the patient begins to loathe food; the pulse becomes very quick but is in general rather weak than strong; the skin feels hot and the patient in the progress of the disorder becomes afflicted with great anxiety and restlessness.

Some likened it to snake poisoning. Such a description would seem to apply to those acute cases in which the toxins elaborated by the infecting organisms were potent and rapidly absorbed, as in gas gangrene or acute hemolytic streptococcal gangrene, or else to those chronic debilitating diseases in which a simple wound or boil becomes secondarily contaminated with gangrene organisms.

The observations of others seemed to indicate that it was purely a local disease without any general manifestation of illness or with only late development of constitutional disturbances. This would seem to apply to relatively mild self-limiting cases or to those treated early.

Most observers considered it primarily a local disease caused by the establishment of the infection in a fresh wound or an old sore, with secondary constitutional disturbances developing either rapidly or slowly. This confirmed the earlier concepts of Thomson and of Blackadder.

Apparently all of the acute and chronic gangrenous infections and progressive ulcerative lesions were included in the category of hospital gangrene, and this fact explains the variety of description and the varied theories regarding etiology.

Guthrie[27] reported a case as follows: 'Postmortem examination, instituted shortly after death, showed the tissues of the limbs and in many cases those of the internal organs also, to be filled with gas and loaded with serous fluid.' This was almost certainly a case of gas gangrene. Other cases characterized by disintegration of the muscle tissues without gangrene of the skin also probably fall into the gas-gangrene group. For example, the case described as follows:

The skin over the injured thigh and buttocks appeared sound, whilst the cellular and muscular tissues of the thigh and buttock were in a state of gangrene, presenting blue and green and greenish gray disorganized matter. In this case, the muscular structures were so disorganized that they were crushed by the slightest pressure. Under the microscope, the muscular sub-

oth venous and arterial, is very common, and in some cases both directly and ıdirectly becomes a cause of death.[27]

Fusospirochetal gangrene may be represented by the following:

In some cases the progress of the disease is rapid and terrible. The edges of ıe wound become hardened and everted, the surface of the wound rises up ıto a pulpy, ragged, gray and greenish mass. When the sloughs are detached, ıe disease attacks other adjacent structures from day to day, extending its ıvages both in length and breadth, and involving aponeuroses, muscles, blood essels, nerves, tendons, and periosteum, and bones and joints.[27]

So extensive was the cross-contamination among crowded soldiers ı those days that there may well have been many cases that had a ombination of these diseases manifesting symptoms that we never see ›day.

The treatment of hospital gangrene in the time of the Civil War was aried. It is perhaps amply summed up by Jones, as follows:

Whether we regard the local affection as a poisoned wound, in which conıgious poisonous matters are continually generated, and from which the ırrounding living tissues are contaminated, or look upon the destruction of ıe tissues as the result of deficient and perverted inflammatory reaction, the rınciples of the local treatment would in either view be much the same. hese indications are best fulfilled by the liberal and thorough application of ›ncentrated nitric acid to the gangrenous parts.

As this is a painful procedure, the patient as a general rule should be placed nder the influence of chloroform and sulfuric ether. During the insensibility f the patient, the surgeon should carefully examine the wound, and first ɛmove all the gangrenous tissues, using the scalpel and scissors, and causing ıe part beneath to bleed quite freely. All sinuses formed under the skin, or etween the muscles, or in the cellular or areolar tissue, must be freely laid pen, and the dead tissues removed. The entire wound is then to be carefully iped out with a sponge or dry lint, and the concentrated acid applied with brush or mop to the entire surface, and care should be taken that the acid enetrate into all the sinuses and cavities. If any diseased part be untouched r undestroyed by the acid, the disease will recommence and spread from that oint.

After extended observation, I am convinced that the failures with the nitric cid amongst the Confederate wounded were attributable mainly to the neglect f the careful cleansing of the wounds, and the failure in applying the acid eely and thoroughly to the diseased surfaces.

The acid destroys the contagious properties of the gangrenous matter, and ›nverts the diseased surface into that of a simple wound or ulcer.[27]

The general requirements of isolation and the constitutional build-up ·ere recognized by most surgeons, but under the handicaps of war and

crowded hospitals these were not often within the range of possibility.

Such was the situation with regard to surgical infections when Pasteur and Lister came on the scene.

V. LISTER'S CONTRIBUTIONS

Lister's name is associated clearly in the minds of all of the medical profession with the beginning of modern surgery. He is given the credit for laying the foundations of our sterile technic in the operating room, which, if properly carried out, effectively prevents, in the great majority of cases, the establishment of an infection in the field of operation no matter what organ or tissue is involved. Many of our profession do not realize that this technic did not come suddenly but was a very gradual growth, first in the mind of Lister, then in the laboratory, and then in the clinic. And after he had demonstrated to *himself* and *his immediate* staff the truth of his theories, his fellow practitioners opposed his ideas, often with jealous bitterness. He had to overcome this opposition by the constant reiteration and repeated proof of his contentions over a period of years. It is a commentary on the perversity of human nature that the continent of Europe accepted his demonstrations before his own colleagues. His whole professional career is a vivid illustration of the time-proved truth, 'A prophet is not without honor save in his own country and among his own people.'

Lister's early contributions to medical literature, beginning in the year 1853, were in the fields of anatomy and physiology, the most important of these being his studies on the coagulation of the blood. In 1858 he published his observations on 'the early stages of inflammation,' but this treatise has the physiologic rather than the bacteriologic point of view.[30] Nine years later, in 1867, his first paper on the new antiseptic principle appeared.[31-2] Four years more passed before he delivered his first speech on 'the germ theory of putrefaction.'[33] He made his second speech[34] four years later, and six more years elapsed before he reached the culmination of his endeavors, when he gave his famous lecture before the International Medical Congress in London in 1881 on the 'Relation of Minute Organisms to Inflammation.'[35] This period of twenty-three years represents a growth and a development—a groping in the dark, a sailing of uncharted seas, a venture into unexplored fields, with many trials and many errors, but a persistence and a faith that would have daunted lesser men. His accomplishments brought him finally full vindication and an everlasting place of honor in the history of mankind.

Perhaps Pasteur is deserving of greater honor, for his work, also done under great handicaps, and his public demonstrations met violent opposition from some of the leaders of the French Academy of Science. He it

both venous and arterial, is very common, and in some cases both directly and indirectly becomes a cause of death.[27]

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FIG. 1. JOSEPH LISTER AND LOUIS PASTEUR. These photographs have always been an inspiration to the author. (Source of photographs unknown)

was who proved the role played by bacteria in the phenomena of fermentation and putrefaction and postulated their role in the production of disease.[24-6] He furthermore disproved the theory of the spontaneous generation of bacteria and devised media for their rapid growth.

Lister used Pasteur's own technic and confirmed the latter's findings. He therefore accepted Pasteur's conclusions and became a strong advocate of his doctrines. He made direct clinical application of the principles and little by little worked out methods that yielded the desired result—uninfected operative wounds. We, as surgeons, therefore look to Lister rather than to Pasteur as the father of modern surgery.

It is of interest to review these twenty-three years and note the highway that Lister traveled to success, and likewise some of the bypaths that often led him astray. His meticulous technic and his careful observation, his tireless repetition of experiments and his willingness to modify his theories to fit inescapable facts are matters of great interest to us today.

The prevailing notion in the early days of Lister's work was that air itself was the cause of decomposition, fermentation, and putrefaction. Pasteur and Lister were obliged over and over again to present demonstrations to dispel this firmly rooted idea. Lister never failed to give Pasteur credit for making his discovery. He was glad to present his own confirmatory experiments and frequently supported these laboratory results with clinical observations. Note his clear thinking in the following passage:

A beautiful illustration of this doctrine seems to me to be presented in surgery by pneumothorax with emphysema, resulting from puncture of the lung by a fractured rib. Here, though atmospheric air is perpetually introduced into the pleura in great abundance, no inflammatory disturbance supervenes; whereas an external wound penetrating the chest, if it remains open, infallibly causes dangerous suppurative pleurisy. In the latter case the blood and serum poured out into the pleural cavity, as an immediate consequence of the injury, are decomposed by the germs that enter with the air and then operate as a powerful irritant upon the serous membrane. But in case of puncture of the lung without external wound, the atmospheric gases are filtered of the causes of decomposition before they enter the pleura, by passing through the bronchial tubes, which, by their small size, their tortuous course, their mucous secretion, and ciliated epithelial lining, seem to be specially designed to arrest all solid particles in the air inhaled. Consequently the effused fluids retain their original characters unimpaired, and are speedily absorbed by the unirritated pleura.[34]

The real beginning of Lister's work on his new antiseptic principle was in 1864—near the end of the Civil War. That he was stimulated to an interest in this subject by the horrible experience of wound infection during that conflict is doubtful. There was enough horror in the civil practice in England and Scotland in those days. His memorable account of his early discoveries is a thrilling story of keen observation and logical thought. He said in 1876:

In the course of the year 1864 I was much struck with an account of the remarkable effects produced by carbolic acid upon the sewage of the town of Carlisle, the admixture of a very small proportion not only preventing all odour from the lands irrigated with the refuse material, but as it was stated, destroying the entozoa which usually infest cattle fed upon such pastures.

My attention having for several years been much directed to the subject of suppuration, more especially in its relation to decomposition, I saw that such a powerful antiseptic was peculiarly adapted for experiments with a view to elucidating that subject, and while I was engaged in the investigation the applicability of carbolic acid for the treatment of compound fracture naturally occurred to me.

Carbolic acid proved in various ways well adapted for the purpose. It exercises a local sedative influence upon the sensory nerves; and hence is not only almost painless in its immediate action on a raw surface, but speedily renders a wound previously painful entirely free from uneasiness. When employed in compound fracture, its caustic properties are mitigated so as to be unobjectionable by admixture with the blood, with which it forms a tenacious mass that hardens into a dense crust, which long retains its antiseptic virtue.[35]

The technic employed by Lister consisted in operating in a field continuously sprayed with a fine vaporlike cloud of carbolic acid but avoiding drenching of the tissues. At first he used pure carbolic, but

gradually reduced it to 1 to 100. He later increased its strength to 1 to 40, feeling more secure with a somewhat stronger solution. Instruments and ligatures as well as sponges were soaked in the same fluid.

Lister's chief interest, however, was in the infection of operative wounds and the process of wound healing under his antiseptic method of treatment. He sums it up briefly as follows:

If we treat our wounds by means expressly calculated to exclude altogether the entrance into them from first to last of minute organisms, those scourges of former surgery fly away as at the touch of the enchanter's wand, to trouble us no more forever, while septicemia also vanishes, and erysipelas, though more stubborn, is robbed of its terrors—not only, I say, do these diseases, recognized as specific, disappear, but, if the treatment is properly conducted, we get rid of inflammation altogether; and we see wounds that are left with their edges widely gaping, and become occupied by a substantial blood clot, heal, it may be, without a particle of pus, a cicatrix forming beneath the superficial layers of coagulum.[37]

Let us mention one or two of Lister's mistakes. The spontaneous development of acute abscesses in the deep tissues puzzled Lister and he was led to believe that they were not caused by bacteria but by some derangement of physiology with secondary invasion by bacteria. He stated as late as 1881:

. . . the inflammation that precedes the suppuration may be induced by some altogether accidental circumstances. For instance, a woman during lactation, with the mammary gland in a state of high physiological activity, corresponding with that of the digestive apparatus of a person after a hearty dinner, as before referred to, is disposed to 'take cold' in the part, and as the result of an accidental chill an acute attack of inflammation may occur, threatening milk abscess. If we get such a case to treat in the early states, the inflammation may never go on to suppuration at all; it may terminate by resolution. But if left to run its course it causes abscess. We can hardly suppose that the accident of exposure to cold could lead directly to the development of micrococci in the part. Nor, even if this were admitted, can we readily understand how any treatment that we can adopt could lead to their dispersion if they were the essential cause of the inflammation.[37]

Occasionally he had noted that what he thought was a pure strain of bacteria grew differently and performed different functions when cultivated in the same medium and he drew an analogy that time has shown to be largely erroneous. He said:

If the same bacterium may, as a result of varied circumstances, produce in one and the same medium fermentative changes differing so widely from each other as the formation of lactic acid and that of black pigment in milk, it becomes readily conceivable that the same organism which under ordinary

circumstances may be comparatively harmless, may at other times generate products poisonous to the human economy. We can understand, for instance, a thing that has at an earlier period of my practice as a surgeon often puzzled me, though now happily, under the antiseptic system of treatment, I never have occasion to witness it, viz. the development of hospital gangrene beneath dressings left for a long time unchanged, whereas in the same hospital ward sores dressed daily continued healthy. Assuming what analogy leads us to suspect, that some organism is the cause of the disease, why should the special virus of hospital gangrene become introduced into a sore under the former condition more than under the latter? We now see that it is not essential to assume the existence of a special virus, at all, but that organisms common to all the sores in the ward, may, for aught we know, assume specific properties in the discharges long putrefying under the dressing.[27]

Although his great interest lay in the healing of compound fractures without infection he conceived the idea of opening abscesses without permitting secondary or, as he thought, primary contamination with micro-organisms.

This plan was based like the treatment of compound fracture, on the antiseptic principle, and the material employed is essentially the same—namely, carbolic acid, but differently applied in accordance with the difference of the circumstances.

In compound fracture there is an irregular wound, which has probably been exposed to the air for hours before it is seen by the surgeon, and may therefore contain in its interstices the atmospheric germs which are the causes of decomposition, and these must be destroyed by the energetic application of the antiseptic agent. In an unopened abscess, on the other hand, as a general rule, *no septic organisms are present*, so that it is not necessary to introduce the carbolic acid into the interior. Here the essential object is to guard against the introduction of living particles from without, at the same time that a free exit is afforded for the constant discharge of the contents.[28]

The application of the antiseptic principle had a profound effect on the general health of the inmates of the hospital and the professional personnel. This effect is admirably described by Lister. He said:

Previously to its introduction, the two large wards in which most of my cases of accident and of operation are treated were amongst the unhealthiest in the whole surgical division of the Glasgow Royal Infirmary, in consequence, apparently, of those wards being unfavourably placed with reference to the supply of fresh air; and I have felt ashamed, when recording the results of my practice, to have so often to allude to hospital gangrene or pyemia It was interesting, though melancholy, to observe that, whenever all, or nearly all, the beds contained cases with open sores, these grievous complications were pretty sure to show themselves; so that I came to welcome simple fractures, though in themselves of little interest either for myself or the students, because their presence diminished the proportion of open sores among the patients. But

> since the antiseptic treatment has been brought into full operation, and wounds and abscesses no longer poison the atmosphere with putrid exhalations, my wards, though in other respects under precisely the same circumstances as before, have completely changed their character; so that during the last nine months not a single instance of pyemia, hospital gangrene, or erysipelas has occurred in them.[36]

It was a great surprise to me in a careful study of Lister's publications that I was unable to find anywhere a detailed description of a case of hospital gangrene. It was apparently so common and everyone was so well acquainted with it that he did not consider it worth while to describe it. Nor do we find any record of a careful bacteriologic study of these cases. We must not forget that Lister was primarily a surgeon.

Lister found the surgical profession unwilling to accept his teachings, and he began to realize that repetition and reiteration and the passage of time were essential for the propagation of his gospel. He continued to report cases in which he had used the principle, but during all of this time and long afterward he labored under the misconception that spontaneous abscesses were not caused by micro-organisms. He wrote in 1867:

> It is now six years since I first publicly taught in the University of Glasgow that the occurrence of suppuration in a wound under ordinary circumstances, and its continuance on a healthy granulating sore treated with water dressing, are determined simply by the influence of decomposing organic matter. The subject has since received a large share of my attention, resulting in the system of treatment which I have been engaged for the last three years in elaborating. The benefits which attend this practice are so remarkable that I feel it incumbent upon me to do what I can to diffuse them; and with this view I propose to present to the readers of the 'Lancet' a series of illustrative cases, prefacing them with a short notice of the principles which it is essential to bear in mind in order to attain success.[32]

In his presentation before the British Medical Association in Plymouth in 1871 he said:

> Among the causes which have hitherto interfered with the general acceptance of this mode of treatment, by far the most prejudicial is the doubt of its fundamental principle, instilled by various authors who have opposed the germ theory of putrefaction, and who, supposing themselves to be advocating the cause of truth, have not only, as it appears to me, espoused the side of error, but have unconsciously inflicted an amount of material evil upon their fellow creatures such as mere speculative opinion is seldom able to produce. For few medical men in active practice have the leisure to sift and weigh the facts and arguments of such a discussion; yet, if they lose firm faith in the guiding principle of the treatment, the attainment of a full measure of success becomes with them a matter of impossibility. 'Felix qui potuit rerum cognoscere causas' was never more applicable than here.

> Another great cause of failure, and consequently of dissatisfaction with the system, is the want of practical initiation into the treatment. For, greatly as our means of carrying out the principle have improved of late, both in simplicity and in efficiency, mere description seems inadequate to convey a clear idea of the method of employing them. Hence, while there are now scattered up and down in this country and in various other parts of the world, gentlemen who, having witnessed the treatment in our wards, whether as students or as qualified practitioners, are attaining exactly the same kind of results as we do, success seems a rare exception for any who have not had such opportunities.[33]

In order to make his hearers bacteriology-minded he said:

> Gentlemen, that you may get satisfactory results with this sort of treatment, you must be able to see with your mental eye the septic ferments as distinctly as we see flies or other insects with the corporeal eye. If you can really see them in this distinct way with your intellectual eye, you can be properly on your guard against them; if you do not see them you will be constantly liable to relax in your precautions.[34]

This holds true today.

In England Lister met much opposition, but he took great comfort from the fact that even if the British surgeons were loath to accept his principles, his visit to the Continent was a series of triumphs for antiseptic surgery. He found strong advocates in Saxtorph in Copenhagen, Nussbaum in Munich, Thiersch in Leipzig, Enke in Stuttgart, Volkmann in Halle, Bardeleben in Berlin, Hagedorn in Magdeburg In America, antiseptic principles received their first partial acceptance only after Lister himself visited Philadelphia in 1876. Little by little his doctrine spread, but as late as 1882 at a meeting of the American Surgical Association the anti-Listerians were in the majority. England, in 1877, finally began to yield before an unanswerable demonstration by Lister in a series of cases at Kings College Hospital. From that time on, the surgeons of England gradually turned to Lister. The International Medical Congress at Amsterdam in 1879 paid him a great tribute and accepted his philosophy, and in 1881 this same scientific body met in London and there in his own country the final complete acceptance came.[35]

In 1883, the principles of Lister were generally accepted by the leading surgeons of America. Willard Parker, H. B. Sands, Robert Weir, W. T. Bull, and others testified without reservation to their unquestionable importance and the necessity for their universal acceptance. Dr. Sands said:

> An extensive experience in the management of wounds according to Lister's methods and other similar methods has convinced me that their success is mainly due to their agency in preventing putrefaction . . I adopt without reserve the germ theory of putrefaction. . . I can testify to a wonderful im-

provement wrought in the management of wounds by antiseptic surgery. . . Primary union is now the rule where formerly it was the exception, diffuse inflammation and suppuration are rare even after severe injuries; operations once formidable now excite little apprehension, and that dreadful scourge, pyemia, has been nearly abolished. . . Much of the opposition to antiseptic surgery at the present day springs from ignorance and prejudice, while those who have applied its principles most carefully have obtained the largest measure of success and unite in acknowledging its achievements.[39]

Willard Parker pointed out that the antiseptic treatment of wounds and aseptic prophylaxis in operations had many indirect advantages. In 1883, he wrote:

Every one of the many minutiae that are associated with Listerism are of the very greatest benefit in an indirect way by calling the attention of the operator, of the assistants, of the by-standers and of the nurses, all, in short, who are in any way connected with the patient's personal surroundings, to the never-ending necessity of cleanliness[40]

Although surgeons acknowledge Lister as the founder of modern surgery, it was Pasteur who laid the solid foundations upon which Lister could build a superstructure that would stand, and to Pasteur full credit was given at his Jubilee in 1892 in Paris. Lister on that occasion delivered this tribute:

Your researches on fermentations have thrown a powerful ray which has illumined . . . surgery and changed the treatment of wounds from uncertain empiricism too often disastrous to a scientific art surely beneficent Thanks to you, surgery has had a complete revolution which has relieved it of its terrors and enlarged its powers almost without limit.[41]

Pasteur had said in 1878:

Si j'avais l'honneur d'être chirurgien, pénétré comme je le suis des dangers auxquels exposent les germes des microbes répandus à la surface de tous les objets, particulièrement dans les hôpitaux, non seulement je me servirais que d'instruments d'une propreté parfaite, mais, après avoir nettoyé mes mains avec le plus grand soin et les avoir soumises à un flambage rapide, ce qui n'expose pas à plus d'inconvénients que n'en éprouve le fumeur qui fait passer un charbon ardent d'une main dans l'autre, je n'emploierais que de la charpie, des bandelettes, des éponges préalablement exposées dans un air porté à la température de 130 à 150 degrés, je n'emploierais jamais qu'une eau qui aurait subi la température de 110 à 120 degrés Tout cela est très pratique. De cette manière, je n'aurais à craindre que les germes en suspension dans l'air autour du lit du malade, mais l'observation nous montre chaque jour que le nombre de ces germes est pour ainsi dire insignifiant à côté de ceux qui sont répandus dans les poussières, à la surface des objets ou dans les eaux communes les plus limpides[42]

In subsequent years efforts have been made by many men to perfect the details of aseptic and antiseptic technic. Out of these labors have arisen all of the minutiae of preparation now in use in every well-regulated operating room.

As long as surgical infections exist, surgeons must make never-ending efforts to control them and to prevent, by every means available, their occurrence. The goal is not yet in sight, but we know what it is and we believe it to be attainable. It may be described as a time when medical science and art can absolutely prevent bacteria from gaining a foothold in the human body.

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CHAPTER II

Surgical Infections and Their Classifications

INFECTIOUS DISEASES are disorders of the animal body caused by the entrance, multiplication, and metabolic activities of micro-organisms. These activities are at first local at the portal of entry of the micro-organism, but later they may become general if the bacteria or their products are carried off in the circulating fluids to other parts of the body. The activities of the micro-organism may be so similar to the activities of the body cells that they do little or no damage to the surrounding tissues or to distant organs. Or the activities may be strikingly different and arouse certain reactions on the part of the local tissues or the body as a whole. These reactions are called the signs and symptoms of an infection. In many instances it is easy, by means of bacterial culture, to find out what organism or combination of organisms is causing the trouble, but at other times the causative agent is not found, either because it cannot be seen under the ordinary microscope or cannot be cultivated on ordinary culture media. Nevertheless, the course of the disease as well as the signs and symptoms all indicate that such disorders are infectious in nature and are caused either by bacteria, by fungi, or by viruses.

Without knowing the cause of any of these infectious diseases, Hippocrates noted that they had certain characteristics of onset, course, and termination distinguishing one from another, and yet they all had certain features in common that made it possible to consider them all members of one large group.[1] They could be distinguished from one another also by their response to, or the alteration of their course by, certain forms of treatment. Today we have the added advantage, in most cases, of being able to determine the bacterial etiology in the early stages before the characteristic features of the natural course of the disease have had time to appear. Frequently, therefore we have an opportunity to institute specific treatment early and halt the establishment and progress of the disease.

Although all infectious diseases have certain features in common, each one presents certain other aspects so that in most instances they can be differentiated one from the other. These differences depend to some extent on variations in the biologic activities of the different types of

bacteria causing them, and on variations in the major site and secondary sites of those activities. This treatise, however, will make no attempt to cover the so-called medical infectious diseases but will limit itself to a consideration of ante- and postoperative surgical infections. What are the differences between medical and surgical infections? What makes an infection amenable to surgical therapy?

An infectious disease is amenable to surgical therapy if two major conditions are met. *First, the major focus of infection must be one not likely to resolve spontaneously, thus leaving the part essentially normal; and second, it must be possible to approach and excise, incise, or drain this focus with relatively little harm to the body as a whole.* With regard to the first condition, it may be said that certain organisms enter and gain a foothold in the body and call forth an inflammatory reaction characterized by swelling, redness, and exudation of fluid into the tissues without destruction of those tissues. After a relatively short time the bacteria are destroyed, their poisons are removed or inactivated, the swelling, redness, and exudation of fluid disappear, and the part is restored to normal. This restoration may be spontaneous, owing to the defensive forces of the body or to certain medicines that have been administered. Such diseases are pneumonia, influenza, acute tonsillitis, and many forms of acute lymphadenitis. Certain other organisms enter and gain a foothold in the body and at first call forth a reaction similar to the first type, but their poisonous metabolic products stimulate a greater local leukocytic response and destroy tissue either by direct action or by the thrombosis of blood vessels. Usually the process is fairly well localized, and although poisonous products, and organisms as well, may spread to other parts of the body, they are usually destroyed. Experience has shown that whereas the body has been able temporarily to localize such a process, it cannot overcome the infection nor restore the affected part to normal as in the first group. Experience has also shown that such a focus may be excised or incised and drained, thereby removing the major portion of the infectious material, and although some damage is done by the incision, the good that is accomplished far outweighs the harm that is done, for the body can then overcome the residual infection, rid itself of the dead and dying tissue, and repair the damaged part by means of scar tissue. In some conditions this process may be aided by the general use or local application of certain medicines, but the principal role is played by the surgical procedure and the defensive and reparative forces of the body. Common examples of such surgical infections are acute appendicitis, acute and chronic cholecystitis, pyonephrosis, whose foci may be excised, subcutaneous abscess, suppurative lymphadenitis, or pyarthrosis, whose foci may be incised; empyema of the pleura or pericardium or gall bladder, whose foci may be drained

With regard to the second condition, namely, accessibility or approachability, one may say that in recent years, areas formerly thought to be inaccessible, are being approached by expert surgeons with increasing confidence. Such areas are the brain, spinal canal, the mediastium, and the lungs. On the other hand, bronchial or mesenteric lymph glands are more difficult to reach. The vegetations on heart valves are still unapproachable and suppurative processes of the portal vein or cavernous sinus cannot be surgically approached without doing more harm than good.

Another condition that renders a surgical procedure ineffective or inadvisable is that of multiple lesions. Such an infection is represented by multiple liver abscesses or miliary tuberculosis or some fulminating cases of osteomyelitis. In such cases, to reach all the involved areas by surgery one would have to do more harm than good. In some cases of septicemia, the portal of entry and the distributing focus cannot be found. Surgical exploration for such foci is sometimes warranted but is generally delayed.

Whereas in some cases of tuberculosis it is possible and advisable to remove surgically the major portion of the diseased tissue, in many cases a cure may be effective if the part is put at rest by fixation, as in tuberculosis of the bones and joints. Sometimes the fixation is accomplished by operating directly at the site of the infection, as in the knee joint, and sometimes indirectly, as in the fusion of the laminae of the vertebrae for tuberculosis of the bodies.

*When one notes the bacteria that have been proved to be the causa*tive agents of infectious diseases, one will observe that those causing a diffuse inflammation that has a tendency toward spontaneous resolution are those against which the body either has some general natural or acquired immunological defense or quickly builds it up during the course of the illness. On the other hand, the organisms causing suppuration and necrosis of the tissue, the so-called pyogenic and necrotizing organisms are those against which the body has little if any natural or acquired immunity and against which it can build up none during the course of the infection. However, usually some local wall or barrier is laid down that protects the body as a whole against the spread of the disease. It may be simply the mechanical blocking of veins or lymphatics with thrombi or inflammatory cells and fluid, but in any event it seems to be a valuable measure of defense.

I. CLASSIFICATION FROM THE POINT OF VIEW OF THE FINAL OUTCOME

A. SELF-LIMITING INFECTIONS

From self-limiting infections the patient recovers completely without medical or surgical treatment, or in spite of it. The body is an extraordinary self-regulating and self-restoring mechanism and recovers completely after certain infectious diseases have run their course. Such disorders as the common cold, or mumps, or some of the acute exanthemata fall into this first group. These are self-limiting infections, since the body almost invariably restores itself. Treatment with medicines may allay certain symptoms but in no way changes the course of the illness. This large group makes possible the practice of quackery or fads or cults, which receive credit for cures for which they are in no way responsible. The apparent success of these practices in the self-limiting diseases encourages both patients and practitioners to permit these methods to be used in the management of diseases of the second group, where their failure is inevitable.

B. SERIOUS INFECTIONS REQUIRING TREATMENT

With the second group of infections, the outcome depends largely on the nature of the treatment, the time after onset within which it is administered, and the judgment of those directing therapy. All of the knowledge we now possess of the behavior of micro-organisms and the reaction of the body to them needs to be used. The early determination of the infectious agent is most important because that often clearly indicates a specific line of treatment. Otherwise the measures used may be hit or miss with a delay in the administration of curative therapy. These measures may be either medical or surgical. The correct medical treatment should be given promptly. The correct surgical treatment requires judgment both as to its kind and the time of its administration. The successful outcome may depend on the proper collaboration between internist, surgeon, and bacteriologist. In this group fall conditions such as septicemia, pneumonia with empyema, primary peritonitis, and so forth, and these infections in their more severe and fulminating forms may pass over into the third group.

C. FULMINATING INFECTIONS

In the final group of infections are those that, at present, prove to be fatal or permanently disabling no matter how expertly or how promptly the treatment is given. Such infections as acute staphylococcus pneumonia, retroperitoneal cellulitis, suppurative pylephlebitis

are included. Our present failure in the treatment of this third group is due largely to the limitations of our knowledge of bacteriology and immunology. As our knowledge grows, this group will become smaller and smaller, and will finally disappear when the goal of research in medical bacteriology has been attained—that is, when micro-organisms can be prevented from gaining a foothold and living in the human body.

When we fail in the treatment of an infectious disease, and either *death or extensive destruction of tissues and permanent disability result*, we like to have the satisfaction of knowing that everything that could be done was done, and furthermore that it was done as soon as possible or just at the proper time. How frequently a review of such a case reveals the fact that the bacteriologic diagnosis was not made at the earliest opportunity or that treatment was delayed until the case was hopeless. Certainly there is room for marked improvement in results in the second and third groups, and our present failures offer a challenge to research in the field of bacteriology.

The purpose of this book, largely, is to help surgeons achieve greater success in the care of patients with infectious diseases of the second group and to help researchers in the field of surgical bacteriology narrow down the number of infectious diseases in the third.

II. CLASSIFICATION ACCORDING TO TIME OF ONSET

According to onset, surgical infections may be divided into three main groups, called anteoperative, operative, and postoperative.

A. Anteoperative Surgical Infections

The anteoperative surgical infections include all those infections in which the organisms have gained entrance to the body before any operative procedure. When the patient comes under the observation of the surgeon, contaminating organisms have already passed the first protective barrier and are either potential sources of infection or have already established themselves in the tissues and have called forth an inflammatory reaction on the part of the body. In the first instance the surgeon has an opportunity of using prophylactic measures against infection. In the second, he is faced with the necessity for active treatment.

Anteoperative infections may be further subdivided into two subgroups: (1) those in which the time and portal of entry are known; and (2) those in which the time and portal of entry are not known.

1. *Anteoperative Surgical Infections in Which the Time and Portal of Entry Are Known*

In this group there is usually a break in the surface of the skin or mucous membrane as the result of an accident of which the patient is aware. The accident may of itself be sufficiently serious to bring the patient at once to the surgeon; or it may be one that the patient himself notices, but he trusts in the self-repairing and self-restoring mechanism of the body; or it may be so trivial that the patient either ignores it or is completely unaware of it and recalls the accident only when the signs of inflammation appear. If the accident brings the patient at once to the surgeon, the latter will presumably carry out certain procedures directed toward preventing infection and favoring repair of the damage done. Sometimes these two principles are in opposition and the surgeon may run the risk of infection by closing the wound in order to get primary union, with its quicker restoration and minimal scar. In any event the surgeon should have constantly in mind the possibility of infection in such cases and observe the wound frequently enough to note the development of infection. An experienced surgeon will have taken a culture of the tissue at the time of the first treatment and will know with what organisms he may have to deal if an infection develops. And if it does develop, the primary cultures will of course have to be checked by subsequent ones in order to show which bacteria have gained a foothold and multiplied.

If the accident does not bring the patient to the surgeon until an infection has developed, the experienced surgeon may be able to predict by the nature of the accident, the site of the injury, the clinical appearance, and the history of the course of events as described by the patient, what organism is probably responsible for the infection. However, he will not depend wholly on his own judgment but will check it by a careful bacteriological analysis in order to be able to apply intelligently the most appropriate treatment. This group of surgical infections will be dealt with more fully in Chapter VII.

2. *Anteoperative Surgical Infections in Which the Time and the Portal of Entry Are Not Known*

These infections differ from the preceding largely in that the bacteria have gained a foothold before the patient is aware of it, and the disease has established itself before the surgeon has had any opportunity to watch or modify its course. In general, these cases develop more insidiously than those of the first group. The earliest stages of the process are overlooked by the patient and easily forgotten so that the course of events from the onset cannot be easily described. Irrelevant preced-

ing events are often interpreted as causes. The whole picture is blurred because the attention is not focused on either the time or the place of entrance of the causative organisms. These usually gain entrance from one of the interior invisible body surfaces. Until we know more about the basic etiology of infections of this type, prophylactic measures will not be possible. As the disease progresses, characteristic features indicate the diagnosis, suggest the causative organism, and give a basis for treatment. The infection may be acute or chronic, mild or severe, fulminating or delayed, latent or recurrent. This group comprises about a third of the surgical patients on any general surgical service and includes cases of acute appendicitis, acute or chronic cholecystitis, peritonitis, chronic pelvic inflammatory disease, suppurative nephritis, osteomyelitis, and so on. The so-called focal infections may also be included. The time of entrance of the bacteria is not known, and often the place can be determined only by exhaustive examinations, or cannot be found at all. These cases will be discussed more fully in later chapters.

B. OPERATIVE SURGICAL INFECTIONS

Operative surgical infections include all those in which the organisms gained entrance to the body at the time of and as an immediate result of the operative procedure. Surgery may be considered either directly or indirectly responsible for their development. It is obvious, however, that they may be divided into two subgroups: (1) those that under the best conditions of sterile operative technic should be prevented, and (2) those that are not now preventable.

1. *Preventable Operative Surgical Infections*

In the decades and generations before Lister and Pasteur, almost all operative procedures were followed by some degree of infection. The conditions described in the first chapter of this book demonstrate clearly not only the limited scope of surgery in those days, but also the fear of both patient and surgeon as much for the outcome of the complication of infection, as for the result of the operative procedure itself. The principles of Lister were not accepted without many objections and reservations. Those that today we take as almost axiomatic were only reluctantly adopted, slowly and step by step, over a period of more than twenty years through the persistent reiteration of principles by one man, supported by the mounting experience of many men who had the courage to put the new principles to the test. This meant inconveniences and annoyances to which some surgeons were not willing to subject themselves, such as masks, gowns and gloves, and restricted movement, but the rewards that came in the saving of lives and the satisfaction of successfully accomplishing operations not pre-

viously thought possible were such that more and more surgeons were willing to pay this price. The principles of sterile technic worked out by Lister and his generation, later generations of surgeons have tried to perfect, and now the surgeon who does not develop a sterile sense and carry out all of the steps of preparation and procedure of sterile technic is not only discredited in the eyes of the profession and the public, but may even be held responsible in a court of law if any untoward event occurs that might be due to his failure to live up to accepted and accredited practice. The various steps in the sterile technic will be considered in detail in Chapter III.

The development of a sterile sense is not easily acquired by every member of an operating team. At first it must be conscious, but gradually by training and practice it becomes habitual and subconscious. It must be followed unerringly and invariably all through the operative procedure and not be broken during the preoccupation of a difficult situation in the operation itself. The individuals must be conscious at all times of the size and scope of the sterile field. Nothing must be touched that is not sterile, without an immediate recognition of the fact and a change of gowns or gloves as the case may be. This includes masks slipping down from the nose, the tear of a glove, or the touching of elbows by a visitor peering over the shoulders. The chief of the service or the head of the team is responsible for keeping up the perfection of the technic and he must watch not only himself but also the other members of his team and call their attention to any break. At the same time each member of the team must watch himself and the others and have no hesitation in pointing out a break by other members including his chief. Anyone so reminded should feel under obligation to thank his informant and immediately take steps to correct the mistake and prevent its recurrence.

2. *Nonpreventable Operative Surgical Infections*

Even with all of the modern methods of sterile technic, it is generally agreed that in practice no wound is ever made in the skin into which some organism does not enter. This is especially true in operative wounds of mucous membranes. Surgical instruments and supplies can be sterilized, but organisms can and do enter surgical wounds from many other sources that we have not yet found the means to control. Some of these sources are:

a. Organisms already resident within body tissues
b. Organisms from a deep focus of infection
c. Organisms resident on the surface of normal mucous membrane.
d. Organisms on dust particles and borne by air currents.

A. ORGANISMS ALREADY RESIDENT WITHIN BODY TISSUES

Although many organisms on the surface of the skin may be removed by a number of methods of cleansing or destroyed or inactivated by a number of so-called antiseptics, no method has yet been found to kill, invariably, all of the organisms harbored presumably in the hair follicles and sweat or sebaceous glands and ducts. When a knife passes through the skin and cuts these ducts and glands, the organisms are smeared over the surface of the wound. That these organisms are viable and pathogenic has been demonstrated time and again, for example, by snipping bits of the full thickness of the skin from hairy regions after the skin has been prepared for an inguinal hernia repair. Furthermore, during the course of an operation organisms from neighboring glands and ducts may be discharged on to the skin surface and be transferred to the wound itself. Various measures may be used to minimize this contamination, as will be described in Chapter VI, but a way has not yet been found to close the door against this source of contamination.

Lymph glands draining a focus of chronic infection or a region of constant contamination, such as the mouth or genitals, may contain living organisms that do not usually invade the surrounding tissues, but that may escape into the wound when the glands or lymph channels are cut. The frequent infection of the wound in high amputations above gangrenous extremities has been attributed to the escape of bacteria from cut lymphatics draining the infected area below.

When bacteria are floating in the blood, an incision into uninfected tissue will release organisms from the blood channels in passing and will cause them to localize and favor their growth in the freshly injured area. This condition was observed by the author in a case in one of the wards of the Presbyterian Hospital (New York City). A patient with a long-standing chronic Staphylococcus aureus osteomyelitis was operated upon in the presence of an acute hemolytic streptococcus pharyngitis. A blood culture taken just before the operation proved to be positive for the streptococcus, while cultures of the bone at the time of operation yielded pure *Staphylococcus aureus*. On the day after the operation, both organisms were recovered from the wound.

B. ORGANISMS FROM A DEEP FOCUS OF INFECTION

Infections that occur in the clean tissues through which an incision is made into an infected focus are almost inevitable. Such an infection commonly occurs when a peritoneal abscess is opened, or a lung abscess is drained, or an osteomyelitis is explored. The wound through the overlying soft parts is usually left open, and the discharges bathe its surfaces. In this process some of the bacteria are detained in crevices

long enough to gain a foothold and penetrate for a certain distance. The principal current of flow is toward the surface, but the more invasive bacteria enter the tissue or are carried back by phagocytes. Great numbers of them may be carried into lateral pockets and be prevented from escaping again by a shifting of planes, as often happens in the transperitoneal drainage of peritoneal abscesses or the transpleural drainage of lung or liver abscesses. In such instances the incarcerated bacteria may spread extensively into surrounding tissues and the infection involve the whole pleura or peritoneum. Usually the superficial wound becomes infected for a limited distance on both sides, and the condition is trivial when compared with the underlying focus, but occasionally the infection of the freshly opened tissues is infinitely more grave than was the deeper original focus, which is often walled off. An example of this was a case of gangrene of the abdominal wall following drainage of a peritoneal abscess of appendiceal origin, described by Brewer and the writer.[2] The infection was found to be due to the synergistic action of two species of bacteria, one of which, a microaerophilic nonhemolytic streptococcus, probably came from the peritoneal abscess, and the accompanying hemolytic *Staphylococcus aureus* either from the skin of the patient or from the air. Sometimes undermining ulcers develop because of the activities of a microaerophilic hemolytic streptococcus. These ulcers may spread over the whole abdominal wall and dip down into the deeper tissues along lymphatic routes. The infection may erode into blood vessels or produce amyloid degeneration of liver, spleen, and kidneys unless it can be stopped in time.[3]

Infections of this nature may sometimes be said to be the result of a fault in surgical technic, as when proper care is not taken to prevent the spilling of the contents of the deep focus into the lateral interstices of the wound, or when the tissues are closed by suture, in whole or in part, sufficiently to prevent the free discharge of their contents.

C. ORGANISMS RESIDENT ON THE SURFACE OF NORMAL MUCOUS MEMBRANES

Another group of operative infections not now preventable by sterile surgical technic consists of those in which an incision is made through clean tissue into a viscus having a contaminated surface, and conversely those in which an incision is made from the contaminated surface of a viscus into clean tissues. Up to the present, no antiseptic has been found for mucous surfaces such as the conjunctivae, respiratory and alimentary tracts, vagina, and urethra, comparable in efficacy to those applied to the dry skin, even when application is made directly to the mucous membrane and over a considerable period of time. Bacteria are normally found in myriads near the orifices of the body and also for a

variable distance away. When incisions are made around the mouth, anus, or vagina, countless bacteria are carried into fresh tissues, and of these a certain number gain a foothold and grow, particularly if there is any closure of the wound. It has been generally observed, however, that if the wound is left open severe infection rarely develops, although bacteria are known to be present that in other locations would invariably lead to an extensive infection. This question will be discussed in more detail in the section on the body's resistance to bacteria, Chapter XVI.

It is a common observation that operations through the abdominal wall on the alimentary canal are fraught with grave danger of infection. The danger is more prevalent and more serious if the opening is made into the esophagus or into the lower ileum or large gut than if it is made into the stomach or duodenum, the difference depending upon the difference in the number and quality of the bacteria in these different regions. When bacteria are normally contained within the viscus there is little evidence, in the present state of our knowledge, that their presence or growth affects the body in any way. If, however, they escape into the peritoneal cavity or into the soft tissues, their presence and growth powerfully alter the functions of the body. Elaborate technical maneuvers have been devised to prevent their escape when the gut is operated upon, but these methods are frequently unsuccessful. It does not seem unreasonable to expect that some means of destroying these organisms on the mucous surfaces of the body will eventually be found. All that can be accomplished by our methods of sterile technic is to bring the sources of contamination down to an 'irreducible minimum,' with the recognition of the fact that this minimum should be still further reduced if progress is to be made.

Attempts have been made to prevent the infection of the peritoneum following resection of (or opening of) the gut, by means of the preliminary injection of vaccines, filtrates, or other substances in order to call forth a protective exudate of leukocytes,[4-6] and by various agents, particularly certain of the sulfonamides, to reduce the number of viable organisms in the intestinal canal. Sulfasuxidine is the best of these.[7] Streptomycin per oram may be even better.[8]

D ORGANISMS ON DUST PARTICLES AND BORNE BY AIR CURRENTS

A very frequent source of contamination, not yet under complete control in most operating rooms, is the air. Organisms are sometimes left in an operating room by a previous case, in the discharges from an infected lesion. If these discharges dry before they are removed, air currents may carry them off and waft them as dust around the room. Dust may also come through open doors and windows or be brought

in on shoes and clothing. Bacteria are also discharged continuously by the personnel during quiet breathing and speaking unless they carefully cover both nose and mouth. Frequent moving about of the personnel and the air currents set up by these motions cause ten times as many organisms to be deposited on a blood-agar plate in a given unit of time as when the operating room is empty. Chapter VI will give details regarding the efforts that have been made to minimize air contamination in our operating rooms, but no method has yet been devised to close the door completely to this source of contamination.

C. POSTOPERATIVE SURGICAL INFECTIONS

In this group are included all of those infections in which the bacteria *gain access to the tissues after the operative* procedure has taken place. These are usually considered complications of the operation and are due to altered physiology. These infections may occur (1) in the wound, (2) in the respiratory tract, (3) in the alimentary tract, or (4) in the urinary tract.

The temperature chart is the best indicator of a postoperative infection. One expects a postoperative rise to 101° F. on the day after operation, 100 to 100.6° F. on the second day, and a gradual fall to normal on the fourth or fifth day, but any level above these should make one suspect a postoperative infection. Rises on the first and second day generally indicate a pulmonary involvement. Virulent wound suppuration may manifest itself in the first two days, but the usual wound abscess develops from the fifth to the tenth day. Inflammation of the urinary tract may occur from the fifth to the fifteenth day. Thrombophlebitis with infarct usually occurs, if at all, in the second or third postoperative week. This is similar to the time of development of subphrenic abscess.

1. *Postoperative Infection of the Wound*

If a wound has been left open after an operation, it is subject to contamination particularly at the time of dressings Organisms may come from the air or directly from the nose and throat or the hands of the dresser. Commonly, masks and gloves are not worn for such procedures and it does not take a very long exposure of such a wound to permit some organisms to fall into it. These organisms may or may not find such a wound a favorable environment for their growth. The fresher the wound the more likelihood that they can gain a foothold. It has frequently been shown in experimental animals that organisms employed to contaminate a fresh wound produce an infection while the same or an even larger dose cannot do so when a barrier of granulation tissue has formed. On the other hand, it is not unusual to note that

organisms such as *Ps. pyocyanea* may appear and proliferate rapidly in a wound that has been granulating for some time. Many organisms may of course grow on the surface of a wound without invading the body or interfering to any important degree with wound healing. On the other hand, Hare,[9] Miles,[10] and others have recently shown that frequently the hemolytic streptococcus, coming in most instances from the noses or throats of the professional attendants, secondarily contaminates an open wound and establishes itself as an infection of primary importance. The author has seen and reported numerous instances of chronic undermining ulceration evidently the result of secondary contamination of open lesions or wounds with the microaerophilic hemolytic streptococcus.[11]

An open wound may also be contaminated by movements of the patient and the shifting and dislodging or loosening of dressings. The patient himself may be inquisitive and peek, handle the dressings, or unconsciously scratch or rub the wound either through or under the dressings. Not infrequently poultices are applied over dressings and these may carry in organisms from the surface. The author has seen cases in which this almost certainly occurred. Occasionally the wound dressings become wet with urine or fecal discharge, which carry in organisms. The spilling of food or drink may also contaminate an open wound.

If a wound is sutured, on the other hand, the chance for contamination and infection is infinitely less. The wound margins soon become sealed with fibrin, which later becomes a scab. If such wounds are wet or macerated, for any reason, this seal may be broken and organisms may be carried in. If such a wound is drained, the tract may permit organisms to enter. It has frequently been observed by the author that a hematoma or necrosis of the skin, at first yielding sterile cultures, after a time shows organisms, and the discharge takes on a purulent character.

2. *Postoperative Infection of the Respiratory Tract*

Normally the respiratory tract above the larynx contains many microorganisms. This condition varies in different individuals and in the same individual from time to time, owing in part to the anatomical differences of the nasal passages and to the varying amount of mucous secretion The bacteria that enter with dust particles are for the most part wafted against the wall of the passage, stick in the mucus, and are subsequently discharged with the accumulated material. Below the larynx, the contamination from inspired air is further guarded against by the cough reflex and by the action of ciliated epithelium, which catches and discharges such foreign bodies as these. The increase in

secretion and the loss of the cough reflex under anesthesia, particularly with such inhalation agents as ether, or even as a result of such sedatives as morphine or the barbiturates, permit contamination of a much greater degree. Such organisms, if of a virulent nature may gain a foothold, especially if the lung is rendered atelectatic by a plugged bronchus or by an infarct, or is congested by weak heart action. Similarly their establishment is favored if the lungs are not aerated, because of shallow breathing, which so often accompanies upper abdominal operations, particularly in elderly people. Organisms getting in during the operative procedure are essentially the same as those entering after operation, but the evidence of their presence does not appear until they produce a fever—sometime within the first forty-eight hours. Pulmonary atelectasis may simulate an infection, but the signs and symptoms promptly subside with the removal of the bronchial plug of mucus. Pulmonary infections that develop postoperatively most frequently occur in patients who have an active upper respiratory infection.[12] Furthermore, virulent organisms may be carried in the nose without evidence of any activity until certain physiologic changes have taken place. Methods have not yet been found to render the nasal passages free from micro-organisms, but the avoidance of elective operations on patients with recent colds, the avoidance of irritating inhalation anesthetics, the avoidance of prolonged loss of the cough reflex with deep anesthesia or sedation, and the encouragement to deep breathing are all prophylactic measures of great importance.

3. *Postoperative Surgical Infections of the Alimentary Tract*

These are rare and of relatively minor importance, but when they occur, they are distressing and in some instances serious. The chief ones are tonsillitis, parotitis, and proctitis.

(1) *Tonsillitis* occurs most frequently after an inhalation anesthetic that has resulted in excessive secretion of mucus and the churning of mouth organisms around in the throat, making quiet breathing impossible without the use of an airway. This occasionally traumatizes the tonsils and pillars and subsequently permits the organisms to enter the tonsillar tissues and start an inflammatory process. In recent years, the frequent use of tubes for purposes of aspiration of stomach contents with the Wangensteen apparatus or the deflation of the small intestine with the Miller-Abbott tube has resulted in trauma to the throat in a fair proportion of cases and this has initiated the establishment of an infection in the tonsils. In most cases, however, this infection responds to local treatment as soon as the tube is removed and simple local applications and irrigations are carried out.

(2) *Parotitis.* It is not unusual for one or both parotid glands to be the site of an inflammatory process after an operative procedure. It occurs most frequently in debilitated individuals and in those in whom fluids have been restricted in the immediate postoperative period, when the mouth is very dry. Curiously enough the organism involved is almost invariably the hemolytic *Staphylococcus aureus*, which is by no means the commonest mouth organism. The gland seems to be particularly susceptible to this organism or the latter has a predilection for the gland. Presumably the organism enters through the duct, although some have suggested a hematogenous origin. The patient usually complains of pain about the time that a swelling appears beneath the ear. Within 24 to 48 hours the whole gland usually becomes involved and pus is seen to flow or may be easily expressed from the duct. The other gland frequently follows suit two or three days later. If the flow of pus from the duct is free, the infection may subside within a few days, but in many instances it continues to increase in severity and in a small percentage of cases it may reach alarming proportions and result in septicemia and death.

(3) *Proctitis.* This is almost always the result of frequent rectal treatment, occasionally associated with postoperative ileus. It usually subsides as soon as the mechanical factor is eliminated, but occasionally an ischiorectal abscess forms, which requires surgical drainage.

4. *Postoperative Surgical Infections of the Urinary Tract*

Operations on the pelvic organs, including the rectum, the bladder, and female generative organs, as well as operations for hernia are frequently followed by a spasm of the urinary sphincter and/or a paresis of the bladder musculature. Sometimes this will respond to local hot applications or rectal instillations, but not infrequently relief from the pain of a distended bladder has to be obtained by catheterization. It is impossible to sterilize the urinary meatus either male or female, so that when the catheter is introduced organisms are carried into the urethra for a variable distance and frequently enter the bladder. This organ is lined with epithelium that stretches and flattens out when the bladder is distended but contracts and thickens when the bladder empties. The organisms that are introduced are the ones most common in that region of the body, having been discharged from the bowel, but again surprisingly enough usually only one or two species persist and multiply. These include the *Escherichia* group and the nonhemolytic streptococci. If the bladder is emptied every eight hours, before it becomes distended, the organisms have only a little chance of gaining a foothold, but if the bladder is permitted to fill repeatedly until it stretches, infection is a frequent occurrence. Another factor that favors a cystitis after organisms have been introduced is the position of the internal urethral opening when the patient is lying down. It is then not

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at the most dependent part of the bladder and there is almost always some residual urine in which the organisms grow and produce their irritating metabolic products. These irritate the mucous membrane and permit invasion of the wall by the organisms. In a certain proportion of these cases, the infection spreads up the ureter either through the lumen or by way of the lymphatics and establishes itself in the kidney pelves. In many cases, the infection persists until the patient gets up out of bed and in the erect position completely empties the bladder with each micturition.

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Sterile Technic

SINCE THE acceptance of the Listerian doctrine of operative-wound infection, a complicated series of procedures designed to prevent the entrance of bacteria into operative wounds has been gradually built up. The same principles are or should be applied to such minor procedures as surgical dressings and intravenous or subcutaneous injection of fluids or lumbar puncture.

Every surgeon and every operating-room or dressing nurse must develop a 'sterile sense' and exercise a 'sterile conscience.' A *sterile sense* is a knowledge of the extent and scope of the sterile field and an *immediate awareness when anything unsterile has come in contact with that field.* A *sterile conscience* is the motive force that compels one to correct immediately a break in sterile technic by oneself or to inform any other member of the team of the latter's break in sterile technic. The privilege of calling attention to a break in technic should *also be given onlookers or anyone in the operating room.* This is essential, for a break, if uncorrected, may result in a fatality. It takes time to develop a sterile sense, requiring that the members of the operating team be able to concentrate on their duties and at the same time be sufficiently extraverted to know what is going on in and around the sterile field. *Not everyone can develop it.* At first it demands strict attention, but gradually it becomes second nature. The sterile conscience depends upon a sterile sense and an appreciation of the responsibility one has for the safety of the patient. Any break, either real or suspected, should be corrected immediately by rendering the region in question *sterile again.*

I. THE SOURCES OF OPERATIVE-WOUND CONTAMINATION

In order to prevent bacteria from entering an operative wound, the *possible sources must be known and the* necessary steps taken to block their entrance. The potential sources of organisms in every operative case are (A) the patient, (B) the operating-team personnel, (C) the

visitors in the operating room, (D) the air in and around the operating room, and (E) the operative instruments and supplies.

A. THE PATIENT AS A SOURCE OF BACTERIAL CONTAMINATION

1. *Organisms Resident on the External Surface of the Body*

The human body is constantly coming into contact with all kinds of bacteria that are deposited on the external surface or inserted or aspirated into one or more of the body orifices. The bacteria, to be sure, may have a very transient abode, until they are rubbed or washed off, or they may be retained for some time. On the skin, they may lodge in crevices, cracks, scratches, pores (both those of sweat or sebaceous glands), or around hairs. Fortunately most of these bacteria are non-pathogenic for man and are of no significance, whereas others are unfortunately capable of producing either a trivial infection or a serious one, which may even cause death. These organisms are for the most part associated with dirt. Many of them are in a dried or sporulating state, whereas others may have come from a human source where they had been recently growing It is generally believed that the staphylococci that are very widely distributed throughout nature are the most common inhabitants of the skin and frequently find the sweat and sebaceous materials suitable media for their multiplication. It is a common occurrence for these organisms to set up a colony within a hair follicle, produce poison, and develop a little area of inflammation that may vary in size and importance from a folliculitis to an abscess or a carbuncle. Other kinds of organisms, for the most part, do not enter the intact skin and are only carried in if they happen to be at the site when a break in the skin is made. Individuals who work in close contact with the soil or who are unclean in their habits naturally have a heavier concentration of bacteria of all kinds on the surface of the body than others have. Different parts of the body surface vary considerably in the degree of contamination, the hands and feet, especially around the nails, being highly contaminated as well as the regions around the various orifices, the mouth, the anus, and the vagina. The hairy regions also carry a heavy load and offer the largest pores for the lodgment of organisms.

A. MEASURES FOR PREVENTING OR MINIMIZING CONTAMINATION FROM THE EXTERNAL SURFACE OF THE BODY

It is obvious that the contaminated body surfaces harbor organisms that have to be dealt with when an operative procedure is contemplated. The skin surface can be readily cleaned with a number of grease solvents, such as benzene, ether, alcohol, and various soaps. The superficial

layers of the skin with their accompanying bacteria can be rubbed off with brushes, but this does not destroy the organisms left on the surface. Many investigations have been made over a period of many years in an effort to answer the question: What substances will destroy the remaining organisms on the surface and also penetrate the pores and eradicate the organisms therein? Various groups of chemicals have been used for that purpose—the phenols, the mercurials, the detergents, the halogens, and the dyes. All of these groups are effective to a certain degree in destroying bacteria in the test tube. On this basis, a number of commercial firms have manufactured derivatives of these main groups and, for the most part, on very meager evidence, they have widely advertised and strongly advocated their use as skin 'antiseptics.'[1] The principal attributes that we desire in a skin antiseptic are: (1) quick killing of bacteria on contact, (2) low surface tension to spread over the skin evenly and penetrate into the pores, (3) detergent properties to dissolve the fatty skin secretions, (4) ability to perform these functions in the presence of the skin secretions and at normal skin temperature, (5) nonspecificity and effectiveness in destroying many different types of organisms in concentrations that are not damaging or irritating to the skin, (6) color that will indicate readily the extent of the area treated but not stain sheets permanently. This question will be considered more in detail in Chapter IV.

Such an 'antiseptic' may be applied to the skin after the surface has been thoroughly cleansed. The ideal preparation should render the skin completely sterile so that pieces of the full thickness of the skin, if taken for culture when the incision is made, will yield no growth, and so that no living organisms can come to the surface during the period of operation. Unfortunately, the ideal skin antiseptic has not yet been found. Iodine fulfils most of these requirements, and the water soluble preparations are somewhat more effective than the alcoholic solutions, but still about 25 per cent of full-thickness skin cultures are positive after contact with the iodine antiseptics.

We must admit, therefore, that for the present we are not able to remove completely or destroy all of the organisms on or in the skin. We can only reduce the number and thus minimize the danger from that source. Nevertheless when we perform operations on uncontaminated deep tissues through a skin thus prepared we call it a 'clean' operation and we expect that the body will be able to take care of the organisms that are introduced and heal the wound without evidence of wound infection.

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We must admit, therefore, that for the present we are not able to remove completely or destroy all of the organisms on or in the skin. We can only reduce the number and thus minimize the danger from that source. Nevertheless when we perform operations on uncontaminated deep tissues through a skin thus prepared we call it a 'clean' operation and we expect that the body will be able to take care of the organisms that are introduced and heal the wound without evidence of wound infection.

B. THE TECHNIC OF PREPARING THE OPERATIVE FIELD

The area should be washed with soap and water and shaved within an hour or two of operation, cutting or scratching the skin being avoided. The soap is washed off with alcohol and the skin allowed to dry. Wearing sterile gloves, the first or second assistant systematically wipes with sponges soaked in alcohol (70 per cent by weight)[2] the area of operation and a wide margin of eight to ten inches around it and repeats this procedure with fresh sponges until they are no longer soiled. In a clean case, the first area to be sponged is the incision site, and then by working outward the necessary limits of the field are reached. This procedure is then repeated with ether. The fluids may be reversed in order, but if the ether is used second, it leaves the skin dry and clean. When there is an infected or contaminated area at the operative site, the preparation should be done from without inward. The cleaned area is then painted with 3½ per cent tincture of iodine or one of the water-soluble iodine preparations, such as ethiomine or tetrodine. (These have given a higher percentage of sterile cultures from full thickness skin snips than any of the other skin 'antiseptics.' If the tincture is used, it is allowed to dry. If covered before it is dry, it may cause skin irritation. It should not be removed with alcohol except in red-headed persons or very light blonds, for it is a compromise, and only such individuals are liable to burn. Four towels are then placed around the site of the intended incision. They are usually held in place with towel clips, but in many cases a silk stitch at the corners or along the towel margins will hold the towels in place better, particularly when the region is one that may flex or extend during the operation, such as the neck. A fenestrated sheet is then placed on the patient so as to hang down over the operating table. Near the head it is held up by a semicircular wire that screens the anesthetist. This region may be reinforced with another small sheet. Realizing that the wound may be contaminated by viable organisms coming from the pores of the skin during an operation, efforts have been made to seal off these ducts Contamination from this source is certainly minimized by clipping towels to the skin edge after the skin incision is made. Zephiran will coat the skin and minimize the discharge of these organisms.[3] Vinylite resin painted on the skin may be still more effective.[4]

2. *Organisms on the Internal Surface of the Body*

The alimentary tract is of course heavily contaminated with bacteria The mouth may offer food particles lodged between the teeth as a medium in which bacteria can grow. Also there may be ulcerations or other areas of inflammation where organisms are not only on the sur-

face but also in the underlying tissues. The esophagus is constantly being contaminated by mouth and food organisms, and if there is any retention of food particles in the wrinkles of the collapsed gullet, the organisms may continue to multiply there. The stomach is the site of great bacterial destruction because of the acidity of the normal gastric juice, which is well below the acid death point of most bacteria. The nonhemolytic streptococci are the most resistant to the action of the gastric juice and may be found commonly in the duodenum or upper jejunum. In patients with a low acidity, the mouth organisms are not destroyed but pass on into the intestine unmolested. If there is any ulceration of the stomach, particularly a malignant ulcer, the contamination of the stomach contents with bacteria is increased. Furthermore, if bacteria are swallowed when suspended in fluid, there may not be time for their destruction before they are whisked on into the small gut.

From the duodenum downward the intestinal organisms increase rapidly in numbers. In spite of the sweeping action of the peristaltic movements, the bacterial content from above downward is greatly augmented by the multiplication of bacteria. As the food elements are withdrawn by digestion and absorption, the relative proportion of the bacterial elements increases. When the stool is discharged, it is estimated that a third of its bulk is made up of bacterial bodies. In the colon, however, they are killed off in considerable numbers so that in the stool many are nonviable. The greatest proportion of viable organisms are found in the lower ileum.

The vagina is constantly being contaminated by intestinal organisms coming from the rectum and a variety of organisms from other sources during intercourse. These casual organisms are usually eliminated by the action of Doderlein's bacillus,[5] which is a normal inhabitant. However, certain pathogens may establish themselves and remain as incitants of inflammation or in a carrier state. The commonest of these are the gonococcus, the aerobic beta hemolytic streptococcus, and the anaerobic nonhemolytic streptococcus.

A. Measures for Minimizing Contamination from the Internal Surfaces of the Body

The problem becomes difficult when we attempt to remove the organisms from the mucous membrane of such approachable surfaces as the eyeball and conjunctiva, the nasopharynx, the mouth, and the vagina. These surfaces are more delicate; they cannot withstand the cleaning procedures used for the skin, they are easily injured by many of the 'antiseptics' we apply to the skin; they have wrinkles, crevices, nooks, and crannies where it is difficult to apply any medication, and

the constant secretion of mucus promptly washes off any surface application. It is obvious that in such regions we can reduce the number of contaminating organisms by surface cleansing, but we cannot reduce them to the degree that we can on the skin. When we perform operations in these regions, we call them clean-contaminated operations and expect a higher incidence of wound infection than we do when we operate through the skin.

The difficulties are insurmountable at present when we try to remove the organisms from the alimentary canal. Unsuccessful attempts have been made to sweep the tract clean by repeated administration of such cathartics as castor oil or to destroy the organisms by such chemicals as dyes.

B. THE TECHNIC OF PREPARING THE INNER SURFACES OF THE BODY FOR AN OPERATIVE PROCEDURE

(1) *The Conjunctiva:* On the evening before operations on the cornea or interior of the eye, penicillin in a concentration of 500 units per gram of ointment base is placed beneath the lids. Just before the operation, a drop of argyrol is instilled. This together with the mucus that clings to it is washed out with saline. The neighboring skin is painted with 3½ per cent iodine.[6]

(2) *The Mouth.* Medicinal-grade zinc-peroxide powder in a 25 per cent suspension in water may be effectively used as a mouth wash three or four times a day and swabbed on the operative site or held in contact with it on a pledget of cotton for a half hour before operation. The same procedure should be continued in the postoperative period. The organisms most to be feared are the anaerobic streptococci, the hemolytic streptococci, the fusoform bacilli, and the spirochetes, all of which this agent effectively controls.

(3) *The Alimentary Tract:* Recently, two sulfonamide derivatives, sulfathaladine and sulfasuxidine, which have the property of slow absorption from the gut, have been used to attempt local destruction of the intestinal bacteria.[7, 8] Streptomycin seems to give even better results,[9] but the effect is often transient. Resistant organisms rapidly multiply again. There is some evidence that the coliform group of organisms can be reduced in number, but the bacteria that remain are a major threat to infection. When we operate through the neck or chest on the esophagus, or through the abdominal wall on the stomach and intestine, we must adopt special technical procedures to avoid contamination from those sources, constantly keeping in mind the presence of bacteria within the tract and preventing, as far as possible, their entrance into the field of operation.

(4) *The Vagina:* After the vagina is washed with soap and water and then irrigated with saline, the vagina and vulva are sponged with 3½ per cent tincture of iodine, care being taken to contact every part of the vaginal surface. After five minutes, this is removed with 70 per cent alcohol on a sponge. Merthiolate[10] may be used as a substitute, and although not as antiseptic, it is less likely to irritate the mucous membrane. For some years the author has favored the use of zinc peroxide as a vaginal preparation for hysterectomy and plastic procedures on the vagina and perineum, the organisms most likely to cause infection being those that are susceptible to this agent. It is applied in a 25 per-cent suspension of the medicinal-grade zinc-peroxide powder in distilled water and left in on packing after the operative procedure.

3. *Organisms within the Body Tissues*

Bacteria are also found within the tissues of the patient, but for the most part these are sterile. The chief site of tissue contamination is the lymph glands, particularly those draining an infected surface area or an infected focus within the body. The mesenteric nodes are probably the most frequent site of bacteria because of the frequent injury to the contaminated intestinal surface from rough particles in the food. The lymph glands destroy organisms in great numbers, but some of the intestinal bacteria almost certainly pass through the filtering system and get into the thoracic duct, and thence into the general circulation. It is probable also that intestinal organisms get into the venous radicles of the mesentery and pass on into the liver. The inguinal and cervical lymph glands frequently contain living organisms, for they drain surfaces that may have ulcerations or frequent injuries. Axillary glands are less often involved, unless there are chronic surface infections or ulcerations of the upper extremity or breast.

When we pass through normal tissues to enter a contaminated viscus or drain an infected focus, we run the risk of infecting these overlying tissues.

A. MEASURES FOR MINIMIZING CONTAMINATION AND INFECTION FROM BACTERIA WITHIN THE BODY

Sulfonamides in the form of powder, particularly sulfanilamide, have been tried but have not been generally successful, probably because these drugs are locally inhibited by the exudates. Penicillin is more effective for this purpose and less likely to be inhibited. It is, however, important to determine as soon as possible the bacterial flora represented in the contaminating secretions or exudate in order that specific antibacterial agents may be employed if any are available. In any case, it is important not to attempt to suture any of the overlying tissues.

B. The Operating-Team Personnel as a Source of Bacterial Contamination

The second most important source of contamination against which the sterile technic of the operating room must guard is the operating-team personnel. Doctors and nurses and other workers around the hospital come into contact more frequently than do the laity with all kinds of bacteria, and particularly with human pathogens. There are almost always cases of infection in the wards of a general hospital. These patients are constantly discharging the causative organisms into their environment either from a draining focus or from their mouths or noses. It is not infrequent to see exudates coming through dressings or leaking around them. The organisms then very quickly get on the bedclothes and then on the hands of the patients, whence they are transferred promptly to the mouth or nose. Later, perhaps after multiplication in the nose and throat, they are discharged again into the environment, so that there is a zone of bacteria around every infected patient. Nurses who daily care for the patients come in contact with these organisms very promptly, and it is not long before they are extensively distributed on the clothing and shoes and particularly on the hands of the nursing attendants. Doctors who dress the patients also pick up these organisms on their hands if they fail to wear gloves, on their clothes if they touch the bed, and on their shoes, unless they too are covered. Hands and handkerchiefs transfer them to the nose and mouth, where they may become established and produce an infection or a carrier state.

1. *Nose and Throat of Operating Personnel as Sources of Wound Contamination.*

A number of years ago, the author was asked to investigate a series of operative-wound infections caused by hemolytic streptococci. A search for this organism in and around the operating room revealed *that 33 per cent of the operating personnel were carrying this organism* in a readily recoverable state in the nose and throat, or both. The throat cultures revealed the organism in many of these doctors, nurses, and orderlies; and some of the nose cultures yielded them also. Furthermore, it was found that the great majority of these individuals harbored large numbers of staphylococci in the nose as well. Just after this survey had been made, another hemolytic streptococcus wound infection developed in a hernia repair and it was found that three of the carriers were on the team that had performed the operation. Curiously enough, the patient yielded positive cultures of hemolytic streptococci in his own nose and throat. The problem was then to find out if possible whether the organism found in the wound was similar to the patient's

nose and throat culture or that of a doctor or nurse on the operating team. All of the strains were inoculated into rabbits and agglutinating sera were obtained. It was then conclusively proved by complete absorption of agglutinin tests that the key strain from the patient's wound was identical with the strains from the nose and throat of the 'sterile' nurse and serologically distinct from the patient's own nose and throat strains and that from the throats of the doctors. This identity was also confirmed by sugar-fermentation tests, which showed that the serologically similar strains both belonged to one of the rarer types of hemolytic streptococci.[11-12]

At that time, there was no masking of the nose by any of the nurses and only by a few of the doctors. After that demonstration, rigid masking of both the nose and mouth was insisted upon for all persons entering the operating room, sterile and unsterile individuals, including orderlies and visitors.

It is perfectly true that covering the nose with a mask is annoying until one gets used to it, but in the face of the incontrovertible proof that unmasked noses are the frequent residence of both hemolytic streptococci and staphylococci, it seems to be almost criminal negligence when operating-room personnel fail to mask thoroughly both the nose and throat. This has been stressed in many publications not only by this author but by Staige Davis,[13] Walker,[14] Koch,[15] and others.

A. MEASURES FOR PREVENTING OR MINIMIZING CONTAMINATION FROM NOSE AND MOUTH

Everyone entering the operating room at any time must have both nose and mouth adequately covered. This statement immediately brings up the question: what is an adequate mask? As one goes about visiting operating rooms, one sees not only many individuals without masks but also a very heterogeneous series of masks, varying considerably in effectiveness. Unquestionably the largest number of organisms is discharged from the nose and mouth during sneezing, when an explosive blast carries out huge numbers of droplets and strings of mucus. This has recently been graphically demonstrated by high-speed photography under brilliant lighting (Fig. 1).[16] The next largest number is discharged during speaking, the number varying considerably among different individuals, depending upon the explosiveness of their speech as well as the degree of salivation.

Hirshfeld[17] has carried out some ingenious tests on the effectiveness of masks and has come to the conclusion that a sneeze and even speaking will cause bacteria to pass through any mask that is not impermeable He has recommended that speaking be prohibited during an operation and only quiet breathing be permitted.



Fig. 2. Mask of face. Bacteria-laden droplets sometimes pass through a mask during talking or coughing (Courtesy of American Association for the Advancement of Science and Dr. M. W. Jennison)

layers of fine-meshed gauze. When this mask is worn, the inner layer catches most of the droplets and the bacteria that pass through as dry individual organisms in the expired air become swirled around and are deposited among the cotton balls and do not pass through the outer layer.[17]

Walker[18] and others feel that the only safe mask is one with some impermeable barrier between layers of gauze. Such a mask seems to be the only kind that will prevent all organisms from passing through, but the barrier diverts air currents to the side, where they may carry bacteria out around the mask to the sterile field. This danger may be eliminated by wearing a helmet with an incorporated fine-meshed gauze four-ply area to cover the mouth and nose and a slit provided for the insertion of a barrier of cellophane or photograph film. The helmet must completely cover the head and extend down over the neck so as to be covered by the sterile gown. Such a helmet will not only prevent particles from falling from the hair and neck onto the operating field, but will also divert the bacteria-carrying air currents down into the gown, where the organisms are deposited on the skin or undergarments.

Impermeable masks unquestionably render breathing difficult, but anyone can get used to that after a little experience. Cone[19] has devised

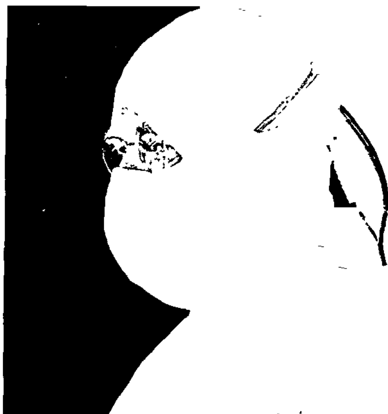


FIG. 3 *Helmet and tube used by the author.* Metal tube under lower lip with an upstanding bridge holds mask away from mouth. Suction carries exhalations through tube down the drain. (Devised by Dr. W. H. Cone of Montreal, Canada)

a simple suction arrangement that obviates this difficulty.* It will be remembered that great objection was made to rubber gloves at first but that now they are accepted without question. Similarly, adequate masks will finally be accepted by all surgeons.

The author cannot stress too strongly the importance of adequate masking during operation and states without fear of contradiction that the nose and throat of operating personnel represent a major source of wound contamination. Failure to mask both nose and mouth by the surgeon is inexcusably reprehensible.

2. *The Hands of the Operating Personnel*

It has been strikingly demonstrated by the author on several occasions that the doctor's hands frequently pick up pathogenic organisms while dressing patients. This is easily proved by requesting the dressing doctor to touch a blood-agar plate with his finger tips directly before and

* This helmet has been used by the author with great satisfaction for several years.

directly after dressing a hemolytic streptococcus case. Even when he has taken ordinary precautions not to touch directly any of the gross exudate or the wound, the hemolytic streptococci will grow out abundantly on the plate. In the great majority of such tests, the plates contacted before dressing will show no hemolytic streptococci and the plates contacted after dressing will show numerous colonies, thus clearly proving that the organisms have been picked up from the patient's surroundings. Cultures from various parts of the body surface and from the bedclothes of a patient with a hemolytic streptococcus infection have shown the extent of this contamination. Hare[20] has abundantly confirmed these findings and demonstrated the spread of the organisms from patient to patient on a ward. Miles[21] and Williams[22] have also stressed this and have devised methods of minimizing this source of contamination during dressings.

It will be remembered that Semmelweis[23] demonstrated that his students carried the organisms of puerperal infection from the autopsy room to the obstetrical clinic and that the contamination could be minimized by the careful washing of hands. Oliver Wendell Holmes[24] similarly proved that the doctors were the chief offenders as carriers of the infections from one patient to another and were chiefly responsible for the puerperal fevers.

It is not surprising that as the winter comes to an end, with alternately cold and warm days, and the hemolytic streptococcus becomes increasingly more prevalent, this species should become established in the nose and throat or both of doctors and nurses and either cause an acute inflammation there or be carried there without causing symptoms. In either event, these organisms then have new foci from which they may be further distributed.

If after dressing a patient, a doctor goes to the operating room without washing his hands thoroughly, it may be assumed with certainty that he is carrying on his hands, clothing, and shoes, and possibly in his nose and throat as well, the organisms that have recently been lodging and growing in a human environment, and that the organisms are in a particularly favorable condition to produce an infection in another patient provided they are planted in an operative wound.

Long before the importance of masking was understood, the more obvious necessity of cleaning the surgeon's hands was apparent. In fact, even in the days of Theodoric, it was deemed advisable for the surgeon to wash his hands.[25] Semmelweis demonstrated in his obstetrical practice the value of hand washing and even of antiseptics.

A. MEASURES FOR PREVENTING OR MINIMIZING THE CONTAMINATION OF THE WOUND FROM THE HANDS OF THE OPERATING PERSONNEL

The preparation of the hands before an operation is called the 'scrub up.' It emphasizes the advisability of scrubbing the surface of hands and forearms with a brush or stiff sponge to take off not only the surface dirt and organisms but also the upper layers of the keratinized epithelium and to open up the ducts of the sebaceous and sweat glands. The time of 'scrub up' is not as important as the vigor used, and yet there is both a practical and an efficient limit, for too long or too vigorous scrubbing may delay operation unduly or damage the skin and thus permit organisms to cling to an abraided surface. In most hospitals, the scrub-up time is either five or ten minutes, either of which is satisfactory from the point of view of removing most of the surface organisms as determined by cultures. The hands are of course dirtier than the forearms and must be given more attention. The nails should first be trimmed and cleaned. A basin for soaking purposes, a good lathering soap, a large brush (not too stiff), and hot water being used, the hands first and then the arms are scrubbed systematically for two minutes, the nails being cleaned again with an orange stick after the free margins are soaped and the hands are softened in the hot water. The rest of the scrub up, from three to eight minutes, should be carried on under running water, the process being repeated over and over systematically from the hands upward to three inches above the elbows. During this process, not only are bacteria largely removed from the surface but some organisms are also washed from the pores and others are discharged from the secretory ducts. It is recognized, however, that no preparation of this kind can be expected to destroy all of the organisms on and in the skin, so that attempts have been made, as yet unsuccessfully, to find some antiseptic that will penetrate the deeper layers of the skin and destroy the organisms lodged there or so coat the surface that any organisms coming to the surface during the course of the operation will be rendered incapable of multiplication. As a result of the teaching of one school, the 'wet-glove technic' is practiced in many places. That is, after the soap and water scrub up, there is usually a washing of the hands and forearms or a soaking with 70 per cent alcohol in a deep vertical or a large horizontal arm bath. Then the gloves, which have been soaking in 1:1000 bichloride of mercury solution, are put on, no effort being made to avoid touching the outside of the glove with the hand. The excess fluid is allowed to run out of the sleeve of the glove, leaving a film of antiseptic on the hands. The gown is then donned and the sleeves of the gloves brought up over the sleeves of the gown. During the operation the skin secretions become mixed with the antiseptic and the organisms presumably are destroyed.

mouth or on the hands or clothing. Unless they are properly covered, they will distribute these organisms around the operating room and run the risk of contaminating the sterile field and the wound. The author has seen, in some of the operating rooms of reputable institutions, unmasked or improperly masked visitors crowding closely around the operating table, peering into the wound and actually touching the sterile elbows of the operator—visitor and operator both apparently unaware of any danger to the patient.

1. *Measures for Minimizing Contamination from Visitors*

A. BALCONIES

Ideally, all visitors and those unnecessary to the performance of the operation should be excluded from the room and supplied with a balcony, protected from the operating room itself by glass partitions, where they can observe the operation with the aid of opera glasses. A microphone and loud speaker may be provided for communication between operator and visitors.

B. GOWNS AND MASKS

If balconies are not available or if for any special reason the visitor must be closer to the operating table, his clothes and arms should be completely covered with a sterile gown. A cap and mask similar to those of the surgeon should be worn, with nose and mouth well covered Speech and movements should be reduced to a minimum.

D. THE AIR AS A SOURCE OF BACTERIAL CONTAMINATION

The bacterial content of the soil and the animals living on the surface of the earth and constantly discharging bacteria into their environment render the atmosphere just above the earth's surface highly contaminated with all kinds of bacteria. The zone of greatest concentration is of course nearest the earth. Pasteur [28] had to go to mountain peaks before he could obtain sterile air. Of course, the degree of contamination is constantly varying because of winds and air currents and the numerical density of animal and human population. Seasonal variations, particularly for certain organisms like the hemolytic streptococcus, are of considerable importance. Temperature, humidity, the amount of sunshine, dust, and rainfall, and other conditions of the weather all play their respective roles in this concentration of bacteria in the atmosphere.

The most prolific sources of organisms that contaminate the air of operating rooms are the people who enter, either before or during the time of the operation.[29] Even though only necessary well-masked personnel enter, bacteria come in on clothing, shoes, stretchers, and blankets. The

patient himself may be an important contributor and usually he is not masked. Previous operations may have left their contributions.

Mas Drets and Madan y Diago[30] found that when the operating team came into the room the normal air contamination was raised thirty-two times. They do not state how well the persons were masked.

The blood agar plates exposed during an operation reveal, after incubation, small dust particles touching the surface of the plate, some of which have given rise to bacterial colonies. The majority of these are staphylococci, both white and yellow, both hemolytic and nonhemolytic. Diphtheroids and *Bacillus subtilis* colonies are frequent, while *E. coli* and nonhemolytic streptococci are only occasionally found. Hemolytic streptococci and *Clostridium welchii* are much more rare. These organisms are frequently cultured from operative-wound infections, and we can be certain that contaminated air is a major source of the organisms.

1. *Measures for Minimizing the Contamination of the Wound from the Air*

For the individual hospital, the problem is to choose for its operating rooms the regions as far removed from bacterial contamination as possible. Ventilation systems are of value if certain principles are observed. Air may be withdrawn from high levels of the atmosphere and passed through oil filters and blown by means of fans through cotton filters into the upper levels of the operating rooms. It then escapes through all of the apertures around doors and windows, so that bacteria are constantly being carried away from rather than toward the operating room, as far as the air is concerned. The air-filtration system may of course be incorporated into an air-conditioning system.

In the early days of antiseptic surgery, Lister[31] considered the air a major source of contamination and attempted to destroy the organisms by a spray of carbolic acid. This spray undoubtedly helped to clear the air, but he admitted that it was not without injury to the tissues, and he gradually gave it up, putting more emphasis on combating other sources of contamination. The dust may be cleared at the beginning of an operation by any kind of sprayed mist, which gradually falls to the floor; but it is the later contamination, when persons are in the operating room, that is more important, and a spray is not practical while the operation proceeds.

Air contamination may be minimized by limiting the number of objects in the room and frequently rubbing down walls and washing the floors with water or an antiseptic such as 1 per cent Lysol. This washing is particularly important after an operation on an infected case during which purulent exudate containing highly virulent organisms may have contaminated the operating table or floor. All the linen from such a

case should be promptly put in laundry bags in which they may be sterilized, and the floors, walls, and furniture should be thoroughly cleaned with an antiseptic such as Lysol.

Air contamination may also be reduced by limiting the number and activity of the persons in the room.

Canopies over sterile instruments and supply tables may be employed to some advantage, but it is hard to protect them from side currents. Robertson's work[32] with the glycol sprays is promising. We may some day have an antiseptic vapor that will be nontoxic, yet effective.

Probably the most potent means we have of destroying organisms in the air at present is the use of ultraviolet radiation. Wells and his co-workers[33] were among the first to demonstrate that bacteria in the air could be destroyed by ultraviolet rays, and Hart,[34] working independently, applied ultraviolet radiation to the air in the operating room. The latter found that the usual precaution of sterile technic on his surgical service did not control infections in wounds of major proportions and in prolonged operations such as thoracoplasties unless ultraviolet generators were employed. Then there was a sharp decline in the number of wound infections, a lowering of the incidence of morbidity as indicated by the postoperative temperature, and an earlier departure from the hospital. He states that he has been able to render the air bacteria-free with intensities that he has found not injurious to the tissues or to the persons in the operating rooms.

Kraissl, Cimiotti, and the author[35] have worked on this problem with the hearty co-operation of the Westinghouse X-ray Corporation. We have carried out extensive experimentation in the laboratory and have evolved an apparatus that seems to answer most, if not all, of the difficulties attendant upon the use of ultraviolet radiation in the operating room. It consists of a Scialitic illuminating unit with the top open so that the heat causes a gentle rise of the air through the center of the lamp and thus a gradual circulation of all of the air in the room. Incorporated into the unit are two semicircular ultraviolet tubes with the focus from the mirrors casting the greatest intensity of radiation about a foot above the patient's wound. Auxiliary units are suspended from the side walls near the ceiling so that the whole atmosphere receives the rays, 80 per cent of which are in the maximum bactericidal zone, with a minimum of those rays that cause erythema. With a radiometer sensitive to these rays of 2,537 Angström units, the exact intensity of radiation may be determined in any square foot of the operating room, and these intensities may be maintained at an even level by stepping up the voltage as the tubes slowly deteriorate. The intensity may be turned down after the operation has proceeded for one hour, and in any event the exposures may be maintained for a duration and intensity well within the safety range.

Under such conditions, we have been able to reduce the bacterial contamination on exposed blood-agar plates in the operating room 90 to 95 per cent, with material reduction, we are sure, in the number of viable organisms entering the wound. There have been no untoward effects observed on the patients. In a series of 75 cases, there was only one infected wound and that occurred in a long operation for carcinoma of the esophagus. A slowly developing anaerobic nonhemolytic streptococcus abscess developed in the wound, whereas the pleura remained free of infection.

Certain precautions must be taken to avoid erythema and conjunctivitis in a long case, namely, a helmet covering face and neck except for a space over the eyes, plain glasses for those who do not use their own, and visors to cut down still further the light from above. One soon gets used to the minor discomforts of this protection. Although our series so far is small, the encouraging results confirming as they do the experience of Hart,[36] Robertson,[37] and others warrant further use of this safeguard against operative-wound infection.

E. Instruments and Supplies as Sources of Bacterial Contamination

A major source of contamination for operative wounds is of course, potentially at least, in the instruments and supplies of linen and gauze, sheets, towels, compresses, pads, and sponges that go into the actual performance of the operation. Contamination from the other sources that we have mentioned—the patient, the operating personnel, and the air—with our present knowledge cannot be completely eliminated, but it is possible to be sure that the instruments and supplies are absolutely *sterile*—and this term in surgery should be used only for the irrevocable death of all bacterial life. The process by which this is brought about is called *sterilization*. It is discussed in the next chapter.

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Sterilization

STERILIZATION MEANS the destruction or removal of all living organisms. It may be brought about in a number of different ways, each of which is applicable to materials used in surgical operative or laboratory procedures.

I. DIRECT FLAME OR INCINERATION

Bacteria are killed promptly when subjected to direct contact with a flame. Burning gases are of different temperatures and for lower levels an extended time must be given. The chief sources of flame around a laboratory or hospital are the incinerator, the Bunsen gas burner, and the alcohol lamp.

The incinerator is used for the destruction of contaminated material of little or no value. It is usually convenient to collect contaminated dressings, sputum cups, and so on, in large paper bags at the bedside of the patient, care being taken to avoid contact with the outside or the margins of the bag. This point should be repeatedly emphasized, because not infrequently the hands of nurses, doctors, maids, and orderlies are contaminated from the dressing bag when this precaution is not observed. In the operating room, contaminated sponges and compresses are usually transferred to bags for incineration, but if it is desired to save them and use them again, they may be sterilized with the sheets and towels in the autoclave.

The Bunsen burner and alcohol flame are used for the quick destruction of bacteria in limited areas on objects not injured by the flame. The hottest part of the flame is at the junction of the inner cone of air with the outer cone of gas. At this point, bacteria are destroyed almost instantaneously. A platinum loop used for bacterial transplants becomes red hot in about a second. Nichrome wire takes a little longer. Glass becomes red hot and then melts in ten to fifteen seconds, depending upon its thickness. The use of a Bunsen burner is of course limited to the neighborhood of the source of gas. It is the flame of choice around a laboratory.

The alcohol flame is more serviceable around an operating room or ward, because it is portable. The flame is very much cooler than that of the Bunsen burner. This fact can be demonstrated easily by comparing the time it takes to heat a platinum or nichrome wire loop or to melt glass in the two flames. The alcohol lamp is useful in heating the lip of a tube or bottle or flask from which sterile material is to be withdrawn, liquid to be poured, or a culture swab to be taken. The container must be held in the flame until the condensed water vapor that forms at the first part of the heating has completely evaporated from the margin of the container. Merely burning alcohol on an instrument that has been dipped in it does not give sufficient time for sterilization. The alcohol flame was frequently used in the operating rooms of Army hospitals in World War I in an attempt to sterilize arm basins and other large receptacles.

II. DRY HEAT

Dry heat is used for sterilizing empty glassware, such as flasks, bottles, Petri dishes, and pipettes that are to be kept dry, as well as various kinds of powders whose chemical and physical state might be altered by moisture. All glassware should be thoroughly cleaned and be free from organic matter. To insure sterilization the degree of temperature and its duration must be sufficient to destroy the most resistant organism that may be present on or in the object to be sterilized. A safe margin for temperature and time is 180° C. for one hour, 170° C. for an hour and a half, 160° C. for two hours, 150° C. for two and a half hours, or 140° C. for three hours.[1] In sterilizing powders, the heat level at which they change chemically or physically must be well known and the temperature must be kept below that point. It is well to keep all dry sterilizing below 190° C., at which level cotton plugs or muslin or paper wrappers are scorched. It is best to plug bottles and flasks with cotton and wrap pipettes and the tops of flasks with muslin or paper securely tied, in order to maintain sterility for some days or weeks after sterilization Bacteriological filters are sterilized by dry heat after proper cleaning and drying. They are set up attached to the tubes or flasks that are to hold the filtrate.

To avoid cracking of glassware, it is important to increase the heat gradually and cool off the glassware slowly. A thermoregulator should be employed to keep the temperature at the desired level, and a self-recording thermometer is of advantage. All material should be distributed loosely, so as to be certain of heat penetration into the innermost part of those materials that are poor conductors of heat. Powders should be sterilized in small portions in flasks or test tubes with cotton-stoppered mouths. Sharp instruments such as knife blades, needles, and scissors

that rust when boiled or autoclaved may be sterilized by dry heat in a similar manner.

Oils and ointments, liquid or solid fats, or other materials that resist the penetration of moist heat should be sterilized in a dry oven. Gauze impregnated with vaseline or zinc-oxide ointment is conveniently prepared in brick-ice-cream boxes of the pint or quart size and should be sterilized in the hot-air oven at the lower temperatures and longer periods mentioned above.

III. MOIST HEAT AT 100° C.

Boiling water is a convenient and satisfactory means of sterilizing surgical instruments, metal utensils, and rubber goods used in the operating room or laboratory. Wet heat will kill bacteria by the coagulation of protein at a lower temperature than dry heat, but the same rule holds that the lower the temperature the more prolonged it must be. Boiling water reaches 212° F. (100° C.) but will go no higher, and a great deal of latent heat is required actually to change water at 212° F. (100° C.) to steam. Many vegetative forms of bacteria are killed at temperatures as low as 130° F. (55° C.) in an hour, or at 175° F. (80° C.) in 15 minutes, this being the usual temperature-time ratio used for killing all of the nonspore-forming organisms in culture media. Above that, the spores of the pathogenic organisms are rapidly killed off—those of *Clostridium welchii*, the common gas-gangrene organism, being destroyed usually in 15 minutes at 185° F. (85° C.) somewhat more easily than the other pathogenic clostridia, which are, however, killed at 195 to 200° F. in 15 minutes. The nonpathogenic spore formers such as *Bacillus subtilis* or *Bacillus sporogenes* are more resistant, frequently resisting boiling (212° F.) for 15 minutes or longer. Boiling for one minute may be expected to kill the ordinary pyogenic organisms, the hemolytic streptococci, the staphylococci, and the colon bacilli, but in the sterilization of utensils and instruments, a sufficient margin of safety must be allowed to kill off the pathogenic spore formers. A safe margin requires a sterilization period of 10 minutes. This time may be shortened or the margin widened if 1 per cent sodium carbonate or 2 to 5 per cent phenol is added to the water.[2]

In the construction and use of boiling-water sterilizers, certain precautions must be taken. There must be adequate protection against contamination of water supply by a back flow from the sterilizers or contamination of the sterilizers by the back flow from the drainage system. This can be easily accomplished by air vents in both the water-supply lines and the drain lines. Instruments placed in the sterilizer should be clean and free from all organic matter, such as blood or pus; clamps

should be open, and the instruments should be completely covered with the water before sterilization begins. The timing of the process should begin only after the water has begun to boil. Vigorous boiling is unnecessary since it only increases steam formation without raising the temperature. The instruments should be contained in a tray with a wire or perforated bottom so that the tray can drain easily and can readily be lifted from the sterilizer and transferred to a sterile supply table without contamination. In the sterilizer, it is important to avoid the nesting of dishes or basins, for this might prevent their contact with the boiling water.

IV. FREE-FLOWING STEAM

Frequently, in laboratories, free-flowing steam is used for the sterilization of media that would be altered physically or chemically by steam under pressure. The various sugars in fermentation media are often so altered and not infrequently precipitates or sediments form under autoclave pressure whereas the media remains clear if it is sterilized under free-flowing steam. The process may be done continuously or intermittently on 3 successive days to insure the sterilization of spores—the media being kept at room or incubator temperature between the periods of sterilization to favor the outgrowth of residual spores into vegetative form.

V. STEAM UNDER PRESSURE OR AUTOCLAVE STERILIZATION

The most effective method of sterilizing the supplies, sheets, towels, sponges, and compresses used for the draping of the operative field and for the staunching of blood during the operative procedure, as well as the final dressings, is by steam under pressure, or autoclave sterilization. Steam under pressure reaches a higher temperature than free-flowing steam, and therefore the sterilization may be accomplished in a shorter period of time. As contrasted with the sterilization of instruments, there needs to be a penetration through many layers of folded linen into the center of a rather large bundle.

One of the most important problems in connection with autoclave sterilization is the removal of air from the chamber of the autoclaves and from the bundles of dry goods. The temperature of pure steam at various pressures is as follows:

5 pounds pressure	109° C. (228° F.)
10 " "	115° C (240° F.)
15 " "	121° C. (250° F.)
20 " "	126° C. (260° F.)
25 " "	134° C. (267° F.)
30 " "	135° C. (275° F.)

If, however, any air is mixed with the steam,[5] the same pressures give *very much lower* temperatures. It is advisable therefore that a mechanism be provided to withdraw as much air as possible just before sterilization begins. This is done by creating a vacuum of *minus 10 inches of mercury*. In rigid tests, we have clearly demonstrated that the period of sterilization may shortened by prolonging the period of preliminary vacuum. However, too prolonged a vacuum may result in superheating and charring of dry goods. It is important that the autoclave be equipped with not *only pressure gauges but also self-recording thermometers that* give a permanent record of the temperature all through the period of sterilization.

An opportunity must be given for the complete removal of air from all of the packages. If the packages are wrapped or packed tightly, this may be *a slow* process, therefore, great pains must be taken to avoid *tight* packing.

Air, being heavier than steam, is displaced downward as the steam enters the chamber. There must be an air vent at the lowest part of the chamber to let the air out all during the sterilization period, and here the thermostatic control must be set up to indicate the temperature in the coolest part of the chamber, which must reach the minimum heat requirements detailed below.

The displacement of air downward by steam indicates that there must be a vertical path always available for the air to get out of any of the packages or the objects within the packages. This must always be kept in mind when the bundles or drums are being packed and placed in the *antoclave. Any object with a concavity upward may form an air pocket* that will prevent the replacement by steam and interfere with the development in that area of the necessary temperature. Such objects might be upright flasks, cups, gloves with cuffs folded back or with fingers pointed downward, or folded rubber sheets.

It has been repeatedly demonstrated by accurate potentiometer tests[6] that large tightly packed bundles or drums with linen in horizontal layers yield the enclosed air and permit penetration by the steam very slowly and incompletely. In spite of the advantages of having all of the linen required for one operation in a single package, large bundles are not safe. Small packages doubly wrapped in fine-meshed muslin *should* be employed and arranged in the autoclave so that all of the ironed surfaces lie vertical and the packages in the different layers are at right angles to one another. If drums are used, the same precautions must be taken with respect to providing vertical avenues of escape for the air.

One of the chief virtues of steam under pressure *is that when it strikes* a cool object like a package of dry goods, it immediately condenses. The

latent heat is then transmitted to the surroundings and the fluid gradually penetrates the package. It takes 865 cc. of steam to make 1 cc. of water. The condensation causes a vacuum, which makes room for more steam to come in. As the condensation of steam repeatedly takes place, the latent heat is given off, and the package soon becomes thoroughly permeated with steam, which at the temperatures noted below rapidly destroys all organisms within the bundle. At the end of the sterilization period, the moisture must be withdrawn from the package by means of the vacuum.

The duration of autoclave sterilization is inversely proportional to the temperature. If all of the air is removed readily, 10 pounds of steam at 240° F. would take approximately 30 minutes to kill all organisms. Fifteen pounds at 250° F. would take approximately 20 minutes. Twenty pounds at 260° F. would take approximately 15 minutes. A margin of safety must, however, be given for the replacement of air in folded packages. A perfectly safe margin for general hospital sterilization of dry goods properly packed as described above in a standard autoclave properly equipped with pressure controls, thermostatic regulators, and air vent would be double the time just noted, namely one hour at 240° F., 40 minutes at 250° F., and 30 minutes at 260° F. The preliminary vacuum should be at minus 10 inches of mercury for 15 minutes and the sterilization time must start when the temperature and pressure have reached the desired level. Any longer periods or higher temperatures are wasteful of time and materials.

Watery solutions may be sterilized in the autoclave without doubling the time and pressures stated above, for no penetration is necessary, because they boil. These sterilization times cover quantities of 2000 cc. or less. Rubber gloves require only surface sterilization, but they should be loosely packed so that there will be no need for penetration. The cuffs should not be folded back and the fingers should point upward so that air pockets will be avoided. The time for rubber-glove sterilization may be the same as for solutions if these precautions are taken.

Frequently in an emergency, it is important to sterilize an instrument quickly. A high-speed autoclave has been designed by Walter [3] that will prepare an instrument in four minutes. I quote from his report:

> Steam is admitted to the jacket of the emergency sterilizer prior to an operation and a steam pressure of 27 pounds [gauge pressure] is maintained until the operative procedure is finished. Thus the sterilizer is instantly available and condensation in the chamber is eliminated. Before placing instruments in the sterilizer, gross dirt and grease must be removed by scrubbing with soap and water and slushing in a fat solvent. The clean fat-free instruments are placed in the sterilizer on a perforated metal tray and the door is

closed tightly. Steam is then admitted to the chamber so rapidly that a sterilizing temperature of 273° F. (134° C.) is attained in 40 seconds. Spores of the most heat resistant organisms can be destroyed at 273° F. in two minutes. . . This technique enables the circulating nurse to return an instrument to the operating table with no compromise of aseptic technique in less than five minutes.

Walter has also devised an autoclave that washes and sterilizes instruments at the same time.

This is done conveniently in a vertical autoclave constructed to withstand an operating pressure of 27 pounds per square inch [gauge pressure]. The dirty instruments are collected in a stainless steel bucket directly from the instrument table. The bucket is placed in the sterilizer over a baffle which forces water to circulate through perforations in the bottom of the bucket. A steam coil located beneath the baffle, supplies adequate heat for rapid sterilization and sets up convection currents in the water to carry the oil and grease, which leave the instruments and rise to the surface, toward an overflow at the rear of the sterilizer. . . The temperature of the water is raised to 273° F. in seven minutes. . . The action of this sterilizer is so rapid and satisfactory that it may be used for the routine sterilization of instruments. . .[4]

Although many well-made autoclaves can be equipped with automatic controls permitting pressure ranges up to 27 pounds, it should be clearly understood that only instruments and utensils can withstand such temperatures. Rubber goods would be quickly destroyed, and fabrics would become charred after a few periods of sterilization. For these the lower temperatures and longer periods outlined above are advisable.

A. Sterilization Controls

Various indicators have been advocated for use in the sterilization process, but they are generally unreliable because, although the melting point is fairly accurate so as to indicate the temperature level reached, they do not indicate how long the temperature was maintained at that level.[7] A better indicator is a resistant spore-forming organism. A strain of *Bacillus subtilis,* which will resist boiling for 15 minutes, is a reliable control for sterilization. A ball of heavy cotton string may be cut into inch-long pieces, which are then saturated with a culture of the organisms and dried. One thread is then wrapped in a double layer of muslin or tied in a small muslin bag. This package is placed in the center of a well-packed drum or between two heavy bundles and in the neighborhood of the air vent at the bottom of the chamber. When the sterilization process is over or when the spore package is removed from the drum, it is sent to the laboratory for transfer to culture media. The ready growth of any

viable organism will quickly reveal any fault in the sterilization process. An added safeguard, which minimizes the human factor in autoclave sterilization, is the time lock devised by Walter,[8] which is released only after a given period of the desired temperature is complete.

VI. BACTERIOLOGICAL FILTRATION

Bacteria may be removed from liquids by filtering through any one of a number of unglazed porcelain filters. The Birkfeld or the Chamberland filters are perhaps the most commonly used. They are in general applicable to relatively small quantities of fluid in which the active ingredients are soluble and which in many instances are injured or destroyed by heat. It is usually necessary to use some form of suction to draw the material through the filters. If the fluid to be filtered is not clear, it is usually advisable to centrifuge it down and filter only the supernatant fluid. After the filtration process, the filtrate must be tested for sterility before it is used. The filter itself must be boiled and then the surface scrubbed and water drawn back through the filter to clean the pores and to bring out any particles that may have penetrated the surface. The filter is then dried and sterilized by dry heat before it is used again.

VII. CHEMICAL STERILIZATION

Certain materials used in the operating room are easily injured or destroyed by boiling or autoclaving, especially sharp instruments such as knife blades, needles or scissors, buttons, dermal or silkworm-gut sutures, and tubes of nonboilable catgut or other suture material. Various chemicals have been advocated for the sterilization of these materials, among them 1:1000 bichloride or biniodide or oxycyanide of mercury, 5 per cent phenol, picric acid, potassium permanganate, merthiolate, mercurochrome, and 70 per cent alcohol (by weight) alone or with 3 per cent formalin and some reducing agent such as sodium nitrite. Most of these solutions fail to give complete sterility. The nearest approach is with the Bard-Parker germicide (3 per cent formalin in 70 per cent alcohol and sodium nitrite).[9] This germicide, however, has its drawbacks because it gives off a gas, thus reducing its formalin content, and the gas is irritating to the hands and mucous membranes of those using it. The solution must also be thoroughly washed off the instruments with sterile water or saline before they are used, in order to avoid the fixing or necrotizing action of the formalin. In spite of these drawbacks, this agent is recommended as the best chemical at present available for this purpose.

The sterilization methods described in Sections I and VII of this chap-

ter have been briefly summarized to bring out the general principles of well-known procedures. Further details may be obtained by consulting the catalogues or brochures of the various manufacturers. The recent developments in air sterilization are, however, not generally known, so that we shall now discuss in detail our own work in this field with respect to the sterilization of the air in the operating room.

VIII. AIR STERILIZATION

Interest in combating air contamination was stimulated by Wells,[10] in 1934, when he showed that moisture droplets from the nasopharynx do not necessarily fall to the ground, but remain suspended in the air and may be considered responsible for transmission of respiratory disease. The size and viability of the organisms are the important factors. He studied the effect of ultraviolet radiation on certain species of bacteria in the air and found that the bacteria could be destroyed.[11]

The application of ultraviolet radiation for sterilization of air in the operating room was described by Hart,[12] in 1936. He found that the usual precautions of sterile technic did not control infections in wounds of major proportions such as thoracoplastic procedures unless bactericidal ultraviolet generators were employed. He emphasized the employment of ultraviolet radiation, from a source essentially monochromatic, which would have the highest bactericidal and the least erythemal effect.

Over a four-year period Kraissl, Cimiotti, and Meleney[13] studied this problem and worked out a safe and efficient installation for operating rooms The author cautions sugeons who contemplate its use to determine that the radiation has the most effective bactericidal quality, and that the intensity of the radiation is sufficient to reduce materially the bacterial content of the air but not so great over a given period of time as to do tissue damage. The following summary gives the essential features of this study.

A. Physical Considerations

Without entering too deeply into a discussion of physics, it may be well for us to consider briefly the fundamental nature of ultraviolet radiation.[14] It will be at once apparent that the term ultraviolet 'light' is a misnomer, as light is only that part of radiant energy that renders objects visible. The relationship of ultraviolet radiation to visible and infrared rays may be best appreciated by the diagram,[15] shown in the following table, the numerals of which are approximate values.

The physiologic effect of any wave length of radiation is extremely critical. This is best brought out by considering the different colors in the visible part of the spectrum. From one end of the colored spectrum

to the other, the violet rays differ from the red by about 35/100,000 of a millimeter. The rays yielding blue light differ in wave length from the rays yielding green light by only 5/100,000 of a millimeter.[16] So that, when we consider radiations in the zone of shorter wave lengths, it might be expected that rays varying only slightly in length might produce quite varied physiologic results.

TABLE I

SHOWING THE RELATIONSHIP OF ULTRAVIOLET RADIATION TO VISIBLE AND INFRARED RAYS

Ultraviolet	*Visible*	*Infrared*
136 Å to 3,000 Å	3,001 Å to 7,700 Å	7,701 Å to 4,000,000 Å

(An Ångström unit is 1/10,000,000 millimeter)

That there have been differences in the effectiveness of different wave lengths of the ultraviolet spectrum was appreciated at a relatively early date. Barnard and Morgan,[17] in 1903, stated that the most effective bactericidal range lay between 3,287 Å and 2,265 Å. Henri[18] also found that the action was greater as the wave length decreased but that the short rays were less able to penetrate protoplasm. Newcomer[19] narrowed the zone of optimum bactericidal action to the region between 3,000 Å and 2,150 Å. In 1918, Bovie[20] demonstrated that paramecia were best killed at 2,800 Å. Further bactericidal work on various organisms by many observers was summarized in Coblentz' work,[21] in 1935, in which the most effective range was found to be in the shorter wave lengths and rapidly diminished at 3,650 Å. The curve of Ehrismann and Noethling[22] has its peak at 2,537 Å and diminishes to nearly zero at 3,000 Å. Coblentz[23] also studied the erythema production of various wave lengths on the untanned human skin and his curve is shown on the accompanying chart, comparing it with the bactericidal effect of Ehrismann and Noethling (Fig. 1).

B. MEASUREMENT OF ULTRAVIOLET RADIATION

Before any consistent results can be obtained with a physical agent, one must know how much of this agent is being used. Inasmuch as ultraviolet radiation extends over a spectral band, it is necessary to have a method of determining how much radiation is being given off in each of the dominant bands.

A history of the various methods of measurement has been compiled by Laurens.[14] At the present time, there are two main types of methods: (1) those based on biologic or photochemical reactions, which measure a gross intensity, and (2) those that employ accurate physical methods such as described by Coblentz.[24] The latter used a complicated apparatus consisting of a photoelectric cell, a balanced amplifier, and a microam-

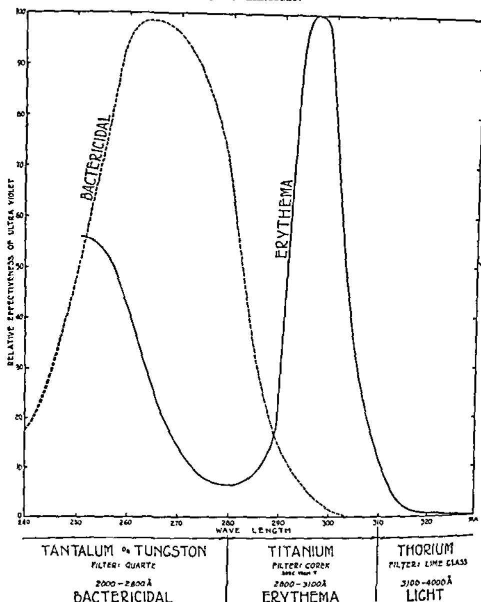


FIG. 1 Curve showing relation of bactericidal effect to the erythema effect (Ehrismann and Noethling)

meter, which will measure minute intensities in the various spectral bands of the sun's radiation.

A simpler form of radiometer has been developed by Rentschler [25] and accepted by the American Medical Association.[26] It has been designed to measure intensities of ultraviolet radiations from artificial sources. By altering the type of glass filter from which the bulb is made and having a metal sensitive to a particular wave-length range, the radiometer is given a selective range and will measure the quantity of radiation in that range. Three cells are available: the thorium, measuring the range from

pig intestine. The animals were operated upon under nembutal anesthesia, and aseptic precautions were observed. The abdomen was shaved and prepared with iodine, which was removed with alcohol. After proper draping, an incision was made and a loop of small intestine, approximately 8 cm. in length, was exposed to the radiations, during which time it was kept moist with saline solution. After exposure, the incisions were closed with three silk sutures through the whole wall and a dressing of collodion applied. The animals that survived were sacrificed after one week, and the viscera were examined.

The best results were obtained with the monochromatic generator. The criterion of adhesion formation was the agglutination of loops of the exposed intestine. Detailed microscopic studies were made of the effect of this radiation upon guinea-pig intestine.

It was felt that it was important to determine just how much radiation of different intensities for varying periods of exposure the viscera could tolerate. The results of this study are recorded in Figure 2. The numerator of the fractions indicates the incidence of adhesion formation and the denominator the total number of animals exposed at the particular intensity and period of time indicated. From this information, a curve of safety was drawn below which it was felt that exposure would not produce adhesions. It will be noted that after one hour, 5 out of 11 control animals developed adhesions although the intestines were kept moist with saline solution during the entire time. Therefore, this was taken as the longest time to expose viscera safely to air without radiation.

E. DETERMINATION OF BACTERICIDAL ACTION

Having determined the safe zone of exposure in terms of intensity and time, it was necessary to plot the previously determined bactericidal effect at these same intensities and times. This was done, using *Streptococcus hemolyticus, Escherichia coli, Staphylococcus aureus, Streptococcus viridans,* and *Bacillus subtilis.* The method was as follows: A blood-agar plate was sprayed with a 24-hour culture of organisms diluted 1:100. the Petri dish was covered with cardboard except for a small square opening in the center, and the exposures were made for the indicated intensity and time. One determination, on *Staphylococcus aureus,* is shown in Figure 3. The end point for this particular determination is six minutes, as the square is seen to have a sharp outline. The central colonies on the seven-minute plate are contaminants. The average of a number of similar experiments indicated complete bactericidal action for *Staphylococcus aureus* to be five minutes at 22 clicks per minute. It will be seen that the lethal points for all organisms fall below the safety line, so that there is an adequate margin of safety. It is evident, therefore, that one may still kill organisms on seeded plates with a lesser

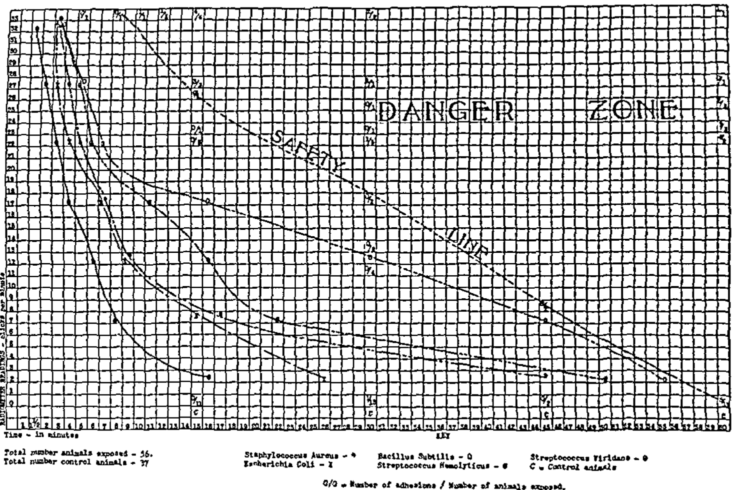


FIG 2 Graph comparing *intensity required to sterilize organisms* on seeded plates with that required to produce adhesions on guinea-pig viscera (1 click = 1.71 microwatts per square centimeter at 1 meter)

mately ten to fifty million organisms per cubic centimeter. It was found that one minute settling time of the bacterial spray in the upper chamber was necessary to remove the fluid part of the culture and prevent droplets from forming on the exposed plates in the lower drawers. The tops of the Petri dishes were opened only after the radiation was discontinued, insuring the fact that the organisms were not destroyed after they had settled on the culture media of the dish. The plates exposed in the control chamber and those exposed in the radiated chamber were then compared.

The intensity of radiation was regulated by altering the distance of the monochromatic generator. It was found that there was not as definite an end point of lethal action in these tests as there was in the exposure of the seeded plate. Some effect was present at as low an intensity as 4 clicks per minute. These tests were performed at different times so that the colony counts on the control plates varied considerably, though we may assume that the number of bacteria falling in the control and in the radiated chambers were the same in any given test. The relationships are shown in the following table.

TABLE III

SHOWING THE EFFECT OF ULTRAVIOLET RADIATION ON BACTERIA SUSPENDED IN AIR

	Test	*Control*
4 c/m	51 colonies in 1 minute	356 colonies in 1 minute
6 c/m	23 colonies in 1 minute	118 colonies in 1 minute
10 c/m	0 colonies in 1 minute	220 colonies in 1 minute
13 c/m	3 colonies in 1 minute	535 colonies in 1 minute
16 c/m	2 colonies in 1 minute	327 colonies in 1 minute

Employing this method of determining bactericidal power in air, variations were made in the time of exposure, time of settling, and intensity of radiation. We found that an intensity of 13 clicks per minute is required to kill bacteria falling a distance of 3 feet in one minute through the air, whereas it takes approximately 9 minutes to kill the same type of organism on the seeded plate. It may be said, therefore, that at this intensity, the radiations are 9 times as effective in air as on the plate. If the intensity is raised to 16 clicks per minute the effect in the air is only 6 times as efficient as on the seeded plate.

F. PRACTICAL APPLICATIONS

Air contamination in an operating room may be determined in a number of ways. A simple method, and one indicative of the contamination of a sterile field as it actually occurs is to expose a blood-agar plate on or near the sterile field of operation. Incubation then will give an indication of what might be expected to fall [illegible] operat[illegible] [illegible]nd.

Some of these organisms may receive further radiation after they have fallen but this may be prevented by a cylindrical guard over the plate. Flushing of the wound with sterile saline and subsequent culturing will give an indication of contamination but will not tell how many organisms will have produced it.

Colony counts made in our operating rooms with exposed Petri dishes showed a predominance of *Staphylococcus albus* and *Staphylococcus aureus*, with hemolytic and nonhemolytic streptococcus, *Streptococcus viridans*, and *Staphylococcus citreus* less frequent.

As has been indicated before, the actual number of colonies varied considerably and depended upon the length of time of exposure, the position of the culture plate, the number of people in the room, and their activity. In one instance, a count of 280 colonies was obtained on one Petri dish in front of a group of students in 30 minutes. In another instance, in a different operating room, there were 66 colonies collected in 165 minutes. An average was made of 10 determinations in one operating room, and it was found that approximately 0.25 colony fell per minute per plate. In another operating room, the average of 74 plates was 1.9 colonies per minute per plate. Averages must be obtained from many tests to show the degree of contamination in any given operating room.

Effort was spent for several years to alter an illuminating unit to include an ultraviolet installation with a fan so placed that it would draw the air upward into the zone of radiation. At the convention of the American College of Surgeons, in October 1937, an operating-room light was displayed that had no cover and made use of a 250-watt bulb with its heat-absorbing filter to promote an upward current of the air. The possibility of utilizing this principle was at once appreciated, and after some time, a unit was developed with an ultraviolet generator built in the lower margin. It was found that the gentle upward current of air would draw any bacteria in the air immediately above the operative wound slowly through the unit so that they might be destroyed while approximating the more intense radiation.

It was apparent that the maximum intensity of the radiation should be above the operative site opposite the exhalations of the operating team, rather than directly on the wound itself. This was accomplished by the design of the reflectors in the illuminating light. At the operative site, the intensity was considered to be optimal at 13 clicks per minute, which has been calculated to kill 99.5 per cent of the organisms in air suspended for one minute. This is about the time an organism will take to drop a distance of 3 feet in still air. With this illuminating unit and its ultraviolet generator, the organisms would be drawn through the zone of intense radiation, and if suspended there longer than one minute, this time would insure their destruction. At this intensity, our experimen-

mately ten to fifty million organisms per cubic centimeter. It was found that one minute settling time of the bacterial spray in the upper chamber was necessary to remove the fluid part of the culture and prevent droplets from forming on the exposed plates in the lower drawers. The tops of the Petri dishes were opened only after the radiation was discontinued, insuring the fact that the organisms were not destroyed after they had settled on the culture media of the dish. The plates exposed in the control chamber and those exposed in the radiated chamber were then compared.

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13 c/m	3 colonies in 1 minute	535 colonies in 1 minute
16 c/m	2 colonies in 1 minute	327 colonies in 1 minute

Employing this method of determining bactericidal power in air, variations were made in the time of exposure, time of settling, and intensity of radiation. We found that an intensity of 13 clicks per minute is required to kill bacteria falling a distance of 3 feet in one minute through the air, whereas it takes approximately 9 minutes to kill the same type of organism on the seeded plate. It may be said, therefore, that at this intensity, the radiations are 9 times as effective in air as on the plate. If the intensity is raised to 16 clicks per minute the effect in the air is only 6 times as efficient as on the seeded plate.

F. PRACTICAL APPLICATIONS

Air contamination in an operating room may be determined in a number of ways. A simple method, and one indicative of the contamination of a sterile field as it actually occurs is to expose a blood-agar plate on or near the sterile field of operation. Incubation then will give an indication of what might be expected to fall on the operative wound.

Some of these organisms may receive further radiation after they have fallen but this may be prevented by a cylindrical guard over the plate. Flushing of the wound with sterile saline and subsequent culturing will give an indication of contamination but will not tell how many organisms will have produced it.

Colony counts made in our operating rooms with exposed Petri dishes showed a predominance of *Staphylococcus albus* and *Staphylococcus aureus*, with hemolytic and nonhemolytic streptococcus, *Streptococcus viridans*, and *Staphylococcus citreus* less frequent.

As has been indicated before, the actual number of colonies varied considerably and depended upon the length of time of exposure, the position of the culture plate, the number of people in the room, and their activity. In one instance, a count of 280 colonies was obtained on one Petri dish in front of a group of students in 30 minutes. In another instance, in a different operating room, there were 66 colonies collected in 165 minutes. An average was made of 10 determinations in one operating room, and it was found that approximately 0.25 colony fell per minute per plate. In another operating room, the average of 74 plates was 1.9 colonies per minute per plate. Averages must be obtained from many tests to show the degree of contamination in any given operating room.

Effort was spent for several years to alter an illuminating unit to include an ultraviolet installation with a fan so placed that it would draw the air upward into the zone of radiation. At the convention of the American College of Surgeons, in October 1937, an operating-room light was displayed that had no cover and made use of a 250-watt bulb with its heat-absorbing filter to promote an upward current of the air. The possibility of utilizing this principle was at once appreciated, and after some time, a unit was developed with an ultraviolet generator built in the lower margin. It was found that the gentle upward current of air would draw any bacteria in the air immediately above the operative wound slowly through the unit so that they might be destroyed while approximating the more intense radiation

It was apparent that the maximum intensity of the radiation should be above the operative site opposite the exhalations of the operating team, rather than directly on the wound itself. This was accomplished by the design of the reflectors in the illuminating light. At the operative site, the intensity was considered to be optimal at 13 clicks per minute, which has been calculated to kill 99.5 per cent of the organisms in air suspended for one minute. This is about the time an organism will take to drop a distance of 3 feet in still air. With this illuminating unit and its ultraviolet generator, the organisms would be drawn through the zone of intense radiation, and if suspended there longer than one minute, this time would insure their destruction. At this intensity, our experimen-

tal animal studies have shown that guinea-pig viscera, which are probably more sensitive than human viscera, will not show changes after 40 minutes' exposure. This would probably exceed the actual time of continuous exposure of any one portion of a patient's viscera during any type of operation.

As has been indicated by Nisbet,[30] and confirmed here, the greatest contamination of the air occurs when the patient is being prepared by the many people who are hurrying about performing their duties, and at the same time stirring up a considerable number of organisms. It was felt that a greater intensity of radiation should be used at this time and gradually reduced as the activity in the room decreased. This would reduce the intensity of the radiation on the exposed viscera and would be adequate to keep the air relatively sterile as the contamination decreased. It has been possible to install a regulating device on the ultraviolet generator that will control the intensity of radiation as required by an automatic time clock, or it may be manually controlled for unexpected prolongation of the operation.

Auxiliary units were placed on the walls in such a manner that sufficient intensity is developed throughout the room to keep the air relatively sterile. Each unit consists of a 30-inch generator with a control to regulate its intensity. It is therefore possible, with the use of a radiometer, to determine accurately the radiation in any part of the room and to raise it or lower it, depending upon requirements.

Such an installation was set up in the laboratory and further bactericidal and adhesion determinations made. For example, 8 animals were exposed at 13 clicks per minute for 30 minutes, and at autopsy, one week later, showed no evidence of adhesion. Bactericidal determinations at different stations in the room were made using seeded-agar plates, similar to those previously described. These findings have been recorded in Figure 2. It was felt, therefore, that this type of installation (Fig. 5) was safe and effective in controlling air-borne organisms and would not injure exposed viscera.

On the bases of these studies, an installation was made in one of the operating rooms at the Presbyterian Hospital, New York. Average colony counts during operation without the ultraviolet radiation were 0.95 colonies per minute. Under similar conditions, the colony count under ultraviolet radiation was only 0.19 colonies per minute. Various types of operations were performed under it. There was a significant diminution of infection rate over a period of one year for both clean and contaminated groups. See Chapter VI.

The operating-room staff, during long operations, must wear tennis visors and clear glass auto-driving goggles to protect their eyes from

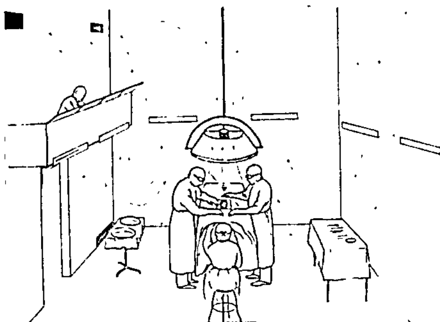


FIG. 5 Installation with automatic intensity control for central unit Note constant intensity over basins and instrument table.

conjunctivitis. Ordinary spectacles do not protect them from the lateral rays. A simple starched helmet seems to be all that is necessary to cover the head, with a safari-type cloth to cover the back of the neck. Some nurses and anesthetists who have failed to cover themselves adequately have complained of nausea and vomiting, but this is rare.

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CHAPTER V

Antisepsis and Disinfection in Relation to Surgical Infections

By Edwin J. Pulaski, M.D.

Antisepsis was not established on a scientific basis until Lister,[1] inspired by Pasteur's work on the micro-organismal cause of fermentation, realized that the catastrophes that in his day regularly followed the treatment of wounds in hospitals were due to infection. The revolutionary benefit conferred by Lister's work followed his demonstration that in the great majority of cases the wounds of any tissue will heal without suppuration or manifestation of putrefaction, provided access of bacteria from without can be prevented. The technic of modern surgical antisepsis has been built on the crude beginning illustrated by Figure 1.

Lister's first reports were on the treatment of compound fractures that had already been contaminated or infected by the time they reached the surgeon's hands. In applying a crude form of carbolic acid (creosote) to the soiled and infected bone and soft tissues with a view of eliminating infection, he was the first to attempt a rational chemotherapy. The chemotherapy of wound infections, whether with carbolic acid or the other agents available at the time, even when the intervention took place early, did not achieve uniform success. Lister himself deprecated bringing carbolic acid into contact with tissues. Since the discovery by Pasteur of the bacterial etiology of infections, the literature has been replete with substances recommended for their antiseptic and disinfectant properties, and each year the list is augmented by the introduction of many new ones.[2] These constitute repeated efforts to improve on Lister's phenol antisepsis.

The field of antisepsis and disinfection has exhibited growth of tremendous scope and complexity. An enormous and widely scattered literature has resulted from the advances in our knowledge of basic facts of the action of antiseptics and from changing concepts in the approach to achieving sterilization of wounds brought about by the introduction of the sulfonamides and antibiotics. In spite of the important role of antisepsis and disinfection in surgery, this phase is one of the neglected subjects in medical curricula. Many substances are used because of tra-

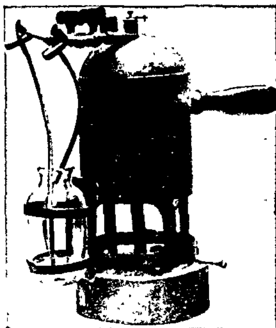


FIG. 1. Large steam spray used by Lister. (Courtesy of the Director of the Wellcome Historical Medical Museum)

dition and habit, and if not for this reason, because of current popularity. There is broad misconception as to what can be done by antiseptic substances to bacterial flora in tissues. The widespread use of certain of these substances to sterilize instruments and excreta has given rise to the frequently expressed belief that all antiseptics are protoplasmic poisons.

I. MODE OF ACTION OF ANTISEPTICS[3-5]

A. DEFINITION OF TERMS

Disinfection is commonly used synonymously with 'sterilization.' It means that any object, after disinfection or sterilization, contains no more living bacteria, protozoa, or fungi. Common usage has resulted in two terms:

1. Disinfectants, or germicides, are agents that actually destroy bacteria, usually in a short time, that is, they are bactericidal agents; and
2. Antiseptics are agents that prevent multiplication without killing all the bacteria, even though they may kill a large number, that is, they are bacteriostatic.

Thus formaldehyde and phenol are strong germicides, whereas penicillin and the sulfonamides are potent wound antiseptics. Few substances are germicidal in body tissues, so that the term 'disinfectant' is reserved for agents used to sterilize inanimate objects and intact skin, whereas the

term 'antiseptic' is applied to those substances used for the control of infection of the body.

B. Characteristics Desirable in Antiseptics

The ideal antiseptic is one that is powerfully antibacterial against all pathogenic bacteria in that bodily environment where its action is required, but at the same time is completely inactive against the constituents of tissues of the body. The following characteristics are desirable in the ideal antiseptic:

1. The substance must be in a form that will permit easy application and rapid delivery to the area needed Thus it must be soluble in tissue fluids well beyond the antibacterial level, and must have a penetrability sufficient to permit entry through and beyond tissue exudates to where the offending organisms reside. If the substance is applied in a vehicle, such as a solvent or ointment, it must be released on contact with tissues in effective concentrations over such period of time as is required to combat infection At the same time, the vehicle must be inert.
2. Stability on exposure to light, air, wide fluctuations in temperature, humidity, and transportation
3. Ease of sterilization providing the substance is not self-sterilizing
4. Lack of irritation on local application, or adverse reaction on general administration
5. Ready washability from wounds and dressings if locally used
6. Ease of manufacture and low cost

No single antibacterial substance available today meets all these requirements. For the proper selection of those agents suitable to the various needs in surgical practice, it is necessary to understand the chemical, physical, and biological properties of antiseptics. The analysis of the differential effect of chemotherapeutic and antiseptic substances on cellular metabolism is of immediate practical importance. Various laboratory tests have been developed to screen the lethal tissue poisons from those agents that warrant further study. If results of such tests are favorable, confirmation is sought first in the experimental animal, then lastly by clinical trial.

C. Factors That Influence Disinfectant Action

The bulk of available data on antibacterial agents consists of experiments to determine the greatest dilution in which an antiseptic will kill or inhibit a given inoculum of bacteria in broth in a given time, at a specified temperature. Such experiments usually employ phenol as a control of the resistance of the culture used; and the result, denoted as the *phenol coefficient*, is obtained by dividing the maximum inhibitory dilution of the unknown by that of phenol. The action of an antiseptic, in

terms of the data obtained by the phenol-coefficient test, depends on three fundamental factors: the concentration, the time, and the temperature.

1. *Concentration*

Information about the minimum concentration in which an antiseptic will act in vitro is useful and affords a guide to the concentration to be used in wounds. If a concentration greater than the minimum is tolerated, the antiseptic may be successful. Dilution affects phenols. It is imperative that they should be used in concentrations known to be comparable with the possible dilution, for example, by body exudates. On the other hand, progressive dilution causes only a gradual reduction in the velocity of action of many dyes and mercurials.

2. *Time*

Concentration and time are interdependent; if the concentration is increased, the time decreases. It is well known that some antiseptics are rapid and others are slow in their action, and allowances for this variation must be made. The most rapid-acting antiseptics are the halogens (for example, Dakin's solution) and those agents that liberate nascent oxygen, such as the peroxides. In contrast, the heavy-metal salts, the dyes, and the sulfonamides are slow-acting antiseptics.

3. *Temperature*

As a rule, an increase in temperature accelerates disinfection, the increment being small in the halogens and great in the phenols. Practically speaking, this is unimportant, since the temperature of anything applied to the body soon will approximate the temperature of the body.

Other factors of importance in relation to suitability for use in surgery are given below.

4. *Selective Action*

(1) Susceptibility to the action of antiseptics in general differs with bacterial species; spore-forming bacteria and tubercle bacilli, for example, are exceptionally resistant owing to their impenetrability. *Streptococcus fecalis* and the Gram-negative bacteria such as the Pseudomonas group possess considerable resistance to many kinds of antiseptics, however the gonococcus and meningococcus are easily killed. (2) Striking degrees of selective action are noted among *different kinds of antiseptics.* Thus penicillin and gentian violet are strongly inhibitory to staphylococci, whereas most Gram-negative bacteria are highly resistant to their action The Pseudomonas group which flourish in the

presence of many antiseptics, succumb to a dilution of acetic acid that other bacteria can resist. Selective action has important practical application as regards choice of an antiseptic for use against a particular bacterial flora.

5. *Habituation*

Exposure of bacteria to sublethal doses of an antibacterial agent is followed by propagation of resistant and mutant forms, which become increasingly difficult to kill. The change is usually specific, involving no change in susceptibility to other agents. This factor has important therapeutic implications, namely the advisability of a change to another agent if a maximum effect is not being achieved with the current one within a few days Habituation is taken up in greater detail later (see section on Resistant Infections).

6. *Behavior in the Presence of Organic Matter*

Actually, very few of the substances that exhibit great antiseptic activity in vitro retain this property in the presence of body tissues. The organic matter with which an antiseptic has to contend in wounds consists of plasma proteins in solution, particulate matter in the form of blood and inflammatory cells, and foreign bodies such as sequestra and necrotic tissue. It is expedient, therefore, to know how an antiseptic behaves in the presence of such organic wastes, and whether any antibacterial action remains after contact with them.

7. *Action on Tissues in the Body*

All antibacterial substances are toxic to some extent to both bacteria and tissues, but the degree of toxicity for the tissues varies greatly. There is no generally accepted method for testing this toxicity as it applies to the different uses of these antibacterials in surgery. Procedures usually employed include determination of minimal lethal doses by intravenous, subcutaneous, or intraperitoneal routes, and of effects after application to the conjunctivae of animals. From these procedures, we learn the general toxicity of an agent according to the route of administration, and the concomitant effects of absorption on contact with vital and sensitive structures. This information is important for agents administered systemically. For the purpose of local application, the effects that usually matter are those on skin, muscle, connective tissue, and blood elements. The cells of these tissues can be studied histologically in experimental wounds or by tissue culture, and the behavior on leukocytes can be gauged by the direct study in the presence of the antiseptic. We do not know the relative vulnerability of the fibroblasts in tissues. How far the behavior of one type of cell can safely be assumed to be that of

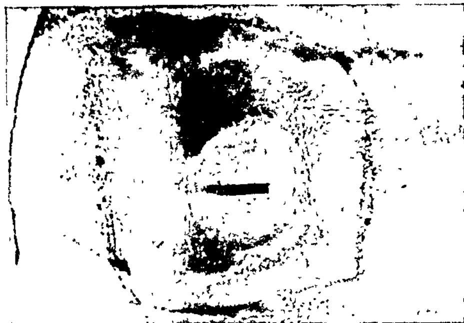


FIG 2 Howes' technique for measuring local toxicity to tissues of test antiseptics. A circular plaque of skin is left on cartilage of rabbit's ear surrounded by a denuded area. Daily photographs indicate growth or inhibition of epithelium from the island. (Courtesy of Dr. E. H. Howes)

another type is not known, but variations are likely. Perhaps the most appropriate test yet devised is the method of Howes[6] for the determination of the effect on wound healing following application of an antiseptic to similar wounds. Howes measures, by serial photographs, the effect on rate of epitheliazation in terms of change in perimeter of an island of epithelium, and the effect on growth of the surrounding granulation tissue. (Fig. 2). Until comparative data obtained by some such method are available, it is difficult to evaluate properly the relative toxicity of antiseptics in wounds, excepting in the case of agents exhibiting gross differences in immediate effects.

8. *Mode of Application*

This introduces two problems: (1) How an antiseptic is to be introduced to a certain area so as to exert its maximum beneficial effects, and (2) how that substance is to be retained in the area long enough to achieve the desired effect. Antiseptics have their greatest antibacterial effect when dissolved in water. However, watery solutions intended for preoperative skin preparation take a long time to dry. The substitution of alcohol as a vehicle ensures more rapid drying on the skin, but has no other advantage. Aqueous solutions of phenol in germicidal strength

(5 per cent) are highly toxic to tissues. The same (5 per cent) concentration of phenol in vaseline is bland and nonirritating to wounds, yet because of the greater affinity of phenol for oil than for water, such an ointment is inert. Generally speaking, the use of any vehicle other than water entails loss of antiseptic power, whether it be used to secure greater solubility, diminish toxicity, or in some way facilitate application. For immediate, rapid effect isotonic watery solutions are best. For more prolonged action a solution of antiseptics is employed in a 'carbowax' water-soluble base, in 'vanishing-cream' water-in-oil emulsions, or in lanolin-type ointments ('Aquafor'). 'Antiseptic ointments' in vaseline are *generally without antiseptic* power. To allow for slowness of release, concentrations higher than those used in aqueous solutions must be incorporated in ointment bases. Comparative data are needed to express numerically the rate of release of antiseptics from different vehicles in comparison with water.[7]

D. RÉSUMÉ OF MODE OF ACTION

The completely ideal antiseptic has not been achieved. Although many substances possess high bactericidal power in vitro, they cannot be looked upon as antiseptics, or, more specifically, as chemotherapeutic agents. One compound may excel in speed of action, whereas another may have superior penetrating ability. For thorough evaluation of all features, there must be a multitude of tests and all results must be carefully considered. It must be determined what degree of concentration of an antiseptic should be used for bacteriostatic or bactericidal action on tissues, how this degree is to be maintained, and over what period of time the substance must remain on the wound surface. Other important considerations are the changes the antiseptic produces in the bacterial flora in the wound, its action on tissue repair, its degree of impotence in the presence of organic matter, and the development of drug resistance. Also the selection of an antiseptic is frequently based on esthetic factors such as odor, color, or staining properties.[8] The last mentioned is chiefly responsible for the reluctance to use dyes in hospitals, because of the added expense involved in laundering soiled linen. These many variables are difficult to control, and data on each are not available. Consequently, it is often impossible to state with certainty that one agent is superior to another. A definite superiority in one or more of the factors mentioned, such as phenol coefficient, low acute toxicity, and so on, probably accounts for the enormous duplication of types of agents constantly being introduced for therapeutic use. It is obvious that some compounds are superior to others. It is tempting to dismiss the subject with the statement that, after all, the criterion for selection is the clinical therapeutic

test. This conclusion is logical, provided that the observations are complete and made on a significant series of patients; and that the observers are cognizant of the many variables that make up any group of 'like' surgical cases.[9]

II. NEEDS FOR ANTISEPTIC AND DISINFECTING AGENTS IN SURGERY

Antiseptic and disinfecting agents are required in surgery to convert, in so far as is possible, non-ideal conditions to ideal; in particular, to prevent the not infrequent irreversible damages associated with implanted bacteria and the resultant infection, and to minimize the damage once infection has occurred. Thus every surgical procedure calls for some measure of antisepsis and disinfection. The following examples are enumerated:

1. Preoperative preparation of the oral cavity when sepsis is present so as to minimize the risk of respiratory disease after inhalation anesthesia
2. Destruction or inhibition of the organisms of the gastrointestinal tract so that surgical procedures may be performed with less danger of infection
3. Prevention or control of infections of the lungs and urinary tract incident to or following surgery
4. Prophylaxis. Wounds accidentally produced are all contaminated after infliction and therefore potentially infected.[10-11] Such wounds frequently occur where ideal surgery is not possible because of such factors as time, experience of the operator, anatomical nature of the wound, and so on. The needs for antiseptic agents are augmented in time of war as adjuvants to the fundamental surgical measures of early excision of infected and devitalized tissue, fixation of injured parts, control of hemorrhage and drainage
5. Treatment of wounds in which bacteria have already gained a foothold locally, and are producing an inflammatory process (local infection)
6. Destruction or inhibition of organisms that have traveled from a portal of entry by means of the blood and lymphatic systems to other parts of the body (generalized infection)
7. Sterilization of linens, sutures, tubes, drains, drug powders, solutions and ointments, destruction of organisms that contaminate sharp instruments, such as knife blades, scissors, and needles, which would be dulled by boiling or autoclaving
8. Sterilization of circulating air in the operating room; elimination of cross-infection in hospital wards
9. Destruction or inhibition of bacteria on the hands and arms of the surgeon, and the skin of the patient at the site of an operation
10. Destruction of organisms contaminating the surgeon's, the nurse's, or the attendant's hands after contact with infected material such as dressings

III. VARIETIES OF ANTISEPTICS AND DISINFECTANTS

These may be classed in three principal groups: the surface antiseptics, characterized primarily by their general chemical activity, the systemic antiseptics, and the physical agents (which have been discussed in Chapter IV).

A. SURFACE ANTISEPTICS: CHEMICAL OR SYNTHETIC AGENTS

1. *Acids and Alkalis*

Most acids and alkalis owe their activity to the liberation in water of hydrogen (H) and hydroxyl (OH) ions. It is postulated that they act by neutralizing vital functional free basic or acidic groups of protein. The resulting metabolic disturbance is lethal to the cells. Some acids, such as acetic and mandelic, have a more selective type of action, which apparently is dependent on the molecule as such. Laboratory data suggest that efficient antisepsis can be achieved in any tissue provided the pH is held below 5 or above 9 for a certain length of time. The means to do it, however, are difficult, or do not find safe employment (except in the urinary tract, where these pH's may be achieved without injury to the epithelial lining, for example, by administration of ammonium chloride, mandelates, phosphates, and so on). This difficulty is due in part to the powerful buffering and neutralizing action of tissues and tissue juices.

The strong mineral acids are too caustic for therapeutic use. Nitric acid, however, is used for the cauterization of superficial dog bites. It forms a firm eschar and does not penetrate too deeply. It permanently stains the tissues yellow. Boric acid is a feeble germicide relatively nonirritating to the tissues. It is used for applications to the eyes and in open wounds such as burns. Boric acid, whether applied to extensive wounds in the form of an ointment or a saturated solution, is a cumulative poison. Its weak antiseptic value suggests that for medicinal use other more active and less potentially harmful agents should be employed.[12] Weak solutions of organic acids, such as acetic, lactic, and propionic are employed for the eradication of superficial Pseudomonas group infections, and as fungicides. In vitro, it takes 10 to 15 minutes for weak acetic acid to kill cultures of the Pseudomonas group. Single applications to wounds are either neutralized by tissue fluids or diluted and washed away by that time. Buffer solutions should be active over a longer period of time and therefore are more effective than solutions of the acid alone. A mixture of 3 per cent acetic acid and 4 per cent sodium acetate (in carbowax base) is more prolonged in its action than a like strength of the acid alone, and is less irritating to tissues.[13] Mandelic acid is an

effective urinary antiseptic. A high degree of alkalinity (if sustained) is antibacterial. Alkali has a destructive and solvent action on most bacteria and other organic matter in wounds. To achieve this effect, continuous applications are necessary, as exemplified by the Carrel-Dakin hypochlorite technic.[14] An excessive degree of alkalinity is objectionable on the grounds that it causes destruction of normal tissue and irritation of the skin.[15] Howes states that wounds in experimental animals tolerate repeated applications of mild acids better than of mild alkalis.[16]

2. *Alcohols*

Ethyl alcohol coagulates proteins and therefore is locally toxic to tissues. This effect is also liable to shield bacteria from its action. (The same applies to ether and chloroform.) The optimal bactericidal concentration of ethyl alcohol is about 70 per cent (by weight); deviations from this strength result in considerable loss of activity.[17] Used as a solvent, it diminishes the efficiency for some purposes of other antiseptics, possibly because of the protein-denaturing effect.[4] Spores can live indefinitely in ethyl alcohol.[18] These undesirable properties condemn the use of alcohols in wounds both as antiseptics and as solvents for implanted dirt and greases. The ineffectiveness against spores renders alcohol unreliable as a means of sterilizing rubber caps of vials on which spore-bearing dust has been accumulating. The wiping of contaminated instruments with a sponge wet with alcohol or a brief immersion in it does not ensure sterility. However, as an agent to reduce further bacterial flora after scrubbing, Hatfield and Lockwood state, 'Through a modification of the Price technique by which the effectiveness of antiseptics *on freshly scrubbed* skin is evaluated, it is concluded that alcohol in strengths of 95 per cent and 70 per cent by weight is preferable to any group of commercially prepared agents specifically designed for skin sterilization.'[19] Acidification (hydrochloric acid 0.5 per cent) is said to improve the germicidal efficiency of alcohol. Isopropyl alcohol, popularized as a result of the wartime shortage of ethyl alcohol, is a somewhat stronger germicide against vegetative forms than is ethyl alcohol.[15] It has, in addition, the advantage of lower surface tension, which enhances its cleansing properties. In contrast to ethyl alcohol, the maximum bactericidal concentration of isopropyl alcohol is *full strength.* (Commercial grades are said to be 98.99 per cent.) The small water content of isopropyl alcohol renders it less corrosive than ethyl alcohol for the sterilization of surgical instruments but, like ethyl alcohol, isopropyl alcohol cannot be depended on to kill spores. Alcohol is used routinely in bedridden patients to prevent bedsores and decubitus ulcers, to harden the skin, and minimize sweating.

3. *Aldehydes*

Formaldehyde, the only aldehyde having significant antibacterial activity, is a gas that dissolves readily in water, and is employed in the form of aqueous solutions. In proper concentration it is effective against all forms of organisms, including spores. Its irritant and coagulent effects make formaldehyde impractical for use directly on the body, but it is of value for the sterilization of inanimate objects (surgical instruments, gloves, excreta, and so on). It is the most reliable agent available for the destruction of spores on contaminated instruments, et cetera. Methenamine, which slowly releases formaldehyde in acid solution, is inert at the neutral or slightly alkaline pH's of wound exudates, and its effects are confined essentially to the urine.

4. *Anionic Types of Antiseptics*

The anionic antiseptics are the neutral or faintly alkaline sodium (or other) salts of acids of high molecular weight, familiar examples being common soap, the ammonium and calcium mandelates, and the 'acid dyes,' of which acid fuchsin is representative. They function through interaction of their acidic ions with the basic groups in bacteria, thereby disrupting a particular enzyme system. Such a reaction is unlikely to take place to any extent in the absence of acid, and hence this form of antisepsis should be effective only at a pH value more acid than that of blood alone. This is confirmed in reports from the literature demonstrating that anionic antiseptics have not been effective in wound exudates, and only moderately useful in skin sterilization. The use of mandelates in acidified urine is the only reliable application of anionics.

5. *Katonic Types of Antiseptics*

Kationic antiseptics are the converse of the anionic. They form a large class, and include the 'basic dyes,' such as brilliant green and crystal violet; the acridines, such as proflavine and 5-aminoacridine; and the colorless aliphatic amines, of which Zephiran, Phemerol, and Ceepryn are examples. They all have one feature in common: they are salts of bases of high molecular weight, and are common hydrochlorides or sulfates. In the majority of cases, they form neutral solutions in water. Several members are highly colored, being, in fact, dyes, whereas others are colorless. Kationics act by combining with essential acidic groups in bacteria. The kation or basic portion of the antiseptic apparently combines with an anion or acidic portion in the bacterium to form non-ionized complexes. The reaction immobilizes these vital ions, which apparently are nucleic acids (which acids constitute 20 per cent of the dry weight of streptococci).

A. THE DYES

These may be roughly divided into five classes.

(1) *The Azo Dyes:* Scarlet Red is the best-known member in this group. It is not an antiseptic but is claimed to have the power of stimulating the proliferation of epithelial cells. Opinions are divided as to the clinical value but 5 per cent Scarlet Red ointment (alone, or with 0.5 per cent oxyquinoline sulfate) is used to stimulate wound healing in burns, superficial wounds, and chronic ulcers.

(2) *The Acridine Dyes:* These include acriflavine hydrochloride ('acriflavine'), proflavine hydrochloride, 'Rivanol,' and 5-aminoacridine, which is nearly colorless. Acridines exert a powerful action against Gram-positive organisms and a moderate action against Gram-negative organisms. Their action is not greatly weakened in the presence of serum. They are bactericidal in strong concentrations and bacteriostatic in high dilutions.[20] Proflavine and 5-aminoacridine are the least toxic to tissues, and they do not inhibit appreciably the phagocytic action of the leukocytes. Therefore, they are the antiseptics of choice. Both have about the same range of biological activity. Proflavine is a yellow dye and is acid in solution (and therefore requires pH adjustment before use), whereas 5-aminoacridine is marketed as a neutral hydrochloride, and it stains neither skin nor clothing. These drugs, unlike the sulfonamides, are not inactivated by p-aminobenzoic acid and their action is uninhibited in pus (except mechanically). They are more active than the sulfonamides against *Clostridium welchii*. Hence they are useful antiseptics, and the British, especially, use them extensively in wounds and for preoperative skin preparation (Bonney's paste).[21] In contaminated and suppurating battle wounds the British recommended highly a 1:100 sulfathiazole-proflavine powder spray.[22-3] Solutions of the acridines are unstable to light and therefore must be kept in amber-colored bottles.

(3) *The Fluorescin Dyes:* These include fluorescin alone, and in combination with the metal mercury, such as mercurochrome. Fluorescin stain has no antiseptic properties but is used in the eye to aid in detecting damage to the cornea, and intravenously, in burns, to determine the depth of burns (under ultraviolet light screen with a Wood filter, third degree burns appear black, whereas second degree burns take on a yellow-green hue).[24] Mercurochrome has been vaunted unduly as an all-purpose antiseptic. Its popularity is due to the appeal of its color, and to the questionable results of early experiments in which its action was tested in acid media.[25] In a neutral or alkaline medium, it is a comparatively weak disinfectant. Numerous experiments have shown that it is incapable of sterilizing skin, superficial wounds, or infected

blood. It can be condemned on the ground of deficient antibacterial power alone.

(4) *The Basic Dyes:* These are exemplified by crystal (gentian) violet and brilliant green, are advocated as surface antiseptics, and are still widely used.[15, 26] Neither type is a strong bactericide, but both are bacteriostatic. Their action is highly selective and primarily against Gram-positive organisms. Strains within species resistance is not uncommon These dyes are practically ineffective against Gram-negative bacteria In wounds, they coagulate proteins, and have only a surface effect. Their application has been in the treatment of infected wounds, such as chronic ulcers, mucous membrances, and serous surfaces. The dye coating of burns has been largely abandoned. The treatment delays skin grafting. The crust of dye and necrotic tissue has the disadvantage of masking early cellulitis and erythema. Dye therapy has the additional disadvantage of being expensive from the standpoint of soilage of linens and increased laundry cost.

(5) *Methylene Blue.* This is but feebly antiseptic and it has been relegated largely to the injection of fistulous tracts, such as the pilonidal and anal, to enhance visualization.

B QUARTERNARY AMMONIUM COMPOUNDS

This series of compounds was introduced by Domagk[27] in 1935, shortly after he had launched Prontosil. Zephiran[28] was the first to be popularized in this country and it has been followed since by Phemerol,[29] Ceepryn,[30] and others. One of the most interesting properties of these kationics is that minute amounts when added to water greatly reduce their surface tension and cause them to foam when shaken. They exert a rapid bactericidal action and are active against a wide variety of organisms. They are sensitive to proteins and as a result of high surface adsorption the antibacterial activity is decreased markedly in the presence of pus cells, tissue debris, blood, and the like. Soaps, which are anionic detergents, inactivate them, and contact with soap must be avoided by careful rinsing of soap-cleansed areas. These frothing kationics are unsuitable for repeated applications to wounds, because their surface activity disorganizes tissue elements, and their antiseptic activity is largely nullified by the protein present.

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of disinfectants, the kationic detergents have little sporocidal activity. They are suitable for the sterilization of surgical instruments and for the storage of sterilized instruments. Sodium nitrite (0.5 per cent) is added to prevent corrosion.

6. *The Halogens*

The rapid germicidal action of chlorine and iodine in water is well known. This action depends on their combination with the amine groups in proteins. Consequently, they are rapidly inactivated by protein, and must be constantly renewed to be effective in wounds. Chlorine is applied usually in the form of hypochlorite (Dakin) solution. Hypochlorite solutions are alkaline, and have the additional advantage that the alkali has a destructive and solvent action on bacteria, and other organic matter, such as pus, necrotic tissue, silk, and catgut. The requirement of constant renewal to obtain antiseptic action in wounds has been met in the Carrel-Dakin method of irrigation[14] which consists in (1) mechanical cleansing, (2) chemical cleansing by Dakin's solution, (3) bacteriologic control, and (4) closure of the clean wound. There is installed in the wound, after thorough mechanical cleansing, a hydraulic system of small, soft rubber tubes, perforated at intervals of 1 cm. from the closed extremity, and so arranged that the fluid will flow equally from each opening. The wound is freely irrigated once every two hours during the night and day with Dakin's solution. Wound dressings are changed daily, and the character of the exudate is studied by stained smears and bacteriological examination. Secondary closure may be done when the exudate becomes bacteria-free. The Carrel-Dakin treatment has several disadvantages: (1) Irrigations must be constantly renewed, special apparatus is necessary, and the daily change of dressings, even if painlessly accomplished, predisposes to recontamination of the wound from outside sources. (2) Surrounding skin must be protected by vaseline gauze, or bothersome irritations will occur. (3) An alkalinity of pH 9 to 10 must be maintained by buffers, because tissue irritation is marked on either side of this range. Thus the solution must be made up freshly (not longer than one week ahead) and kept in dark bottles, because of its photolability. (The U.S.P. preparation is a buffered solution) Chloramine-T, Dichloramine, and Azochloramid are slower in action and slower to combine with organic matter than is Dakin's solution, and possess greater persistence of activity in the presence of serum proteins. These agents are more stable than Dakin's solution. However, they are photolabile also and must be kept in dark bottles. Also they do not possess the lytic effect on slough, manifested by Dakin's solution.

Iodine is one of the most dependable agents for preoperative skin disinfection. It is traditionally used in the form of its tincture. (As

mentioned previously, the only advantage of the tincture is the rapid drying upon evaporation of the alcohol.) But it must not be overlooked that iodine is highly germicidal *in aqueous solution. Nye*[31] *showed that* Lugol's solution (U.S.P.) is active *in vitro* in even a 1:64 dilution. The use of aqueous iodines for preoperative skin preparation is not only feasible but advantageous. Several preparations (Ethiomine, Tetrodine) *were found to be equal or superior to tincture of iodine (U.S P.) in* sterilizing the heavily contaminated skin of guinea pigs as tested by culturing pinch grafts before and after single applications of the agents.[32] These aqueous solutions spread, penetrate, and dry more quickly than Lugol's solution because certain surface active agents are added. On application to abraded tissues aqueous iodines do not cause the stinging sensation common to tinctures. Reluctance to recommend the use of aqueous iodines has been due largely to the necessity of making a choice between proprietary preparations. Certain organic iodines (Chiniofon, Vioform, Diiodoquin) are widely used as amebacides.[8, 15] However, they have no antiseptic value when used as dusting powders on wound surfaces. Iodoform has a penetrating odor, which no doubt has long been associated with the idea of antisepsis. However, it is practically insoluble in water or serum, and therefore practically inert. The belief that iodoform liberates iodine when in contact with tissues lacks modern confirmation. Aristol Powder (thymol iodide) is even less soluble in water than iodoform, and is probably of no value as a local antiseptic.

7. *Heavy-Metal Salts and Compounds*

A. MERCURY

Inorganic mercury salts act on the sulfhydryl (SH) groups of cells, coagulating them to form mercury proteinates.[5] The chemical compound so formed inactivates vital enzyme systems and deprives them of nutritional support. The formation of mercury proteinate is a reversible reaction, and under proper conditions the compound may be dissolved, leaving the bacteria again free to absorb food and to multiply. It is known that skin, blood, and wound exudates contain sulfides and sulfhydryl groups, and in such media nullification of the bacteriostatic action of the mercurials and bacterial revival will take place. Simple mercuric compounds have the additional disadvantage of irritation and toxicity, and their use in wounds is unwarranted. Mercuric chloride solutions (which are acid) are inadequate as hand rinses, because of the slowness of the antibacterial action, and because a small amount of soap will inactivate a whole basin of the solution. The corrosive action on metals precludes the use of simple mercury salts for the sterilization of instruments. A 1:1000 solution of mercury oxycyanide is sometimes used

for the disinfection and storage of mechanically clean catheters, bougies, filiforms, and so on, where contamination by spores is not a danger. Potassium mercuric iodide is alkaline, and has been incorporated in soaps to increase germicidal activity. Potassium mercuric iodide or mercuric chloride does not reduce the flora of the skin appreciably, but paradoxically a 'sterile' cutaneous surface may be produced. This phenomenon, according to Price, is due to the transparent 'film' on the skin, under which bacteria are imprisoned.[17] These conditions are so favorable to life that multiplication takes place, the existing bacterial flora doubling every 50 minutes. When the film is broken up, as by sweating, the bacteria are released uninjured. The position of the complex organic mercurials such as 'merthiolate,' the phenylmercuric compounds, 'metaphen,' and others is not so clear. It may be that they behave as kationic antiseptics, since they possess kations of high molecular weight, and do not readily liberate inorganic mercuric ions.[3,4] These organic mercurials are many times more potent, yet less toxic and less irritating, than simple mercury salts, and are claimed to retain a fair proportion of this activity in the presence of serum proteins. However, blood and pus are severely inimical to their activity and in vitro they are inert in thioglycollate (SH compound) broth media. Thus, like the simple mercury salts, they are not suitable for introduction into a wound. For disinfecting instruments, they are not as efficient as is commonly believed.[33] They are popular preoperative skin disinfectants. However, they are not sporocidal and their rate of action is slower than the kationics or iodine. Merthiolate is unreliable as a preservative for stored liquid plasma.[34] As stated elsewhere, solutions of mercurochrome are not reliable skin or wound disinfectants.

B. SILVER

Silver nitrate is employed in surgery chiefly for the cauterization of wounds, and for trimming of granulation tissue, warts, and so on. Its action on wounds is accompanied by irritation, pain, astringency, and corrosion. The tannic-acid silver-nitrate treatment of burns has been abandoned The method increases the depth of burns, masks cellulitis and infection, and delays wound healing and early skin grafting. Colloidal silver compounds are noncorrosive and relatively nonirritant. Their antiseptic action is due to the liberation of very low concentrations of silver ions. The colloidal silver compounds are used mainly on mucous membranes, for antisepsis. The long-continued use of any silver preparation may produce irremediable discoloration of the skin or mucous membrane (argyria). Solutions of silver compounds are photolabile and should have been recently prepared.

C. BISMUTH

The insoluble bismuth compounds are used for their mechanical action as protectives of inflamed or irritated surfaces. Bismuth compounds with phenols in which bromine or iodine has replaced hydrogen in the benzene ring have an antiseptic action. Plastic surgeons recommend one such compound, bismuth tribromphenate (Xeroform), to cover skin-grafted areas and donor sites.[35] Under such dressings delay in wound healing has not been observed. The formula for Xeroform ointment is:

bismuth tribromphenate 3 vaseline 95 paraffin 1 beeswax 1

Many layers of narrow mesh gauze in an oblong tin box are saturated with the ointment and sterilized in the oven at 140° C. for 2 hours

8. *Oxidizing Agents*

The oxidizing agents include ozone, peroxides, perborates, and permanganates. They act by oxidizing constituents of the protoplasm of bacteria. Anaerobic organisms adversely affected by a high concentration of oxygen in the environment are inhibited by agents of this type. All these substances are rapidly inactivated by protein of any kind, and do not distinguish between bacteria and tissues. Unless they effect instantaneous sterilization they must be used over a long period of time and therefore must be constantly renewed. This disadvantage has been met successfully with the introduction of active zinc peroxide (U S.P. medicinal grade).[15, 36] The powder (freshly prepared in sterile water to make a 40 per cent cream, or in carbowax base to make a 20 per cent strength ointment), when applied to a wound, slowly liberates oxygen for a period of 24 to 48 hours. In vitro, it is bactericidal to sporulating anaerobic Gram-positive bacilli, hemolytic streptococci and anaerobic Gram-negative cocci and bacilli, but not to pneumococci, staphylococci or most aerobic Gram-negative rods. Some of the uses of zinc peroxide in surgery are (1) as a mouth rinse and gargle in Vincent's angina and in pyorrhea alveolaris; (2) on foul-smelling, indolent wounds such as ulcerated cervical cancers, which are covered by sloughs produced by Gram-negative anaerobic nonsporeforming bacilli, for example *Bacillus fusiformis* and *Bacillus necrophorus;* (3) in conjunction with excision and wide exposure for controlling the chronic indolent burrowing ulcers caused by a microaerophilic hemolytic streptococcus,[36] (4) in conjunction with excision of devitalized tissues and wide exposure for controlling the spreading bacterial synergistic skin gangrene caused by the symbiotic action of two or more organisms;[36] (5) in empyema after evacuation and rib resection where anaerobic mouth organisms proliferate on remaining fibrin and adherent slough; (6) as a dressing around colostomies, in

a severe dermatitis.[8] Amphyl, a combination of certain alkyl and halogen phenol derivatives of synthetic origin, is a valuable disinfectant of low toxicity that maintains its germicidal powers reasonably well in the presence of moderate amounts of organic matter.[5] Parachlorophenol is worthy of mention.[38] Meleney, Johnson, Pulaski and Colonna [39] found it most effective against the Pseudomonas and Proteus groups, not only *in vitro* in high concentrations (1:3000 to 1:5000), but also in the control of superficial and adequately exposed wounds infected with these organisms. They recommend a 0.25 per cent concentration in carbowax base applied once daily. The various agents presented in the foregoing paragraphs, many of which still are used extensively, represent continued efforts to improve wound antisepsis. Their inconstant success as 'all-purpose antiseptics' has led to widespread pessimism as to their utility. Two divergent reactions have resulted: first, new antiseptics are always appearing, and there is a great increase in the number used, second, the school has developed that discredits all antiseptics and attempts instead to stimulate the normal reactions of the body against the infective process. This school limits itself to utilizing heat and hypertonic salt solutions (lymphogogues).

B. SYSTEMIC ANTISEPTICS

These modern chemotherapeutic agents act primarily by attacking a specific functional unit of a bacterium. There is obviously a much wider field of utility open to such drugs since they do not merely exert their influence locally, but act within the body through the circulation and are thus able to control a general infection.

Systemic chemotherapy has a long history progressing from the older treatment of syphilis by mercurial inunctions, the control of leprosy by chaulmoogra oil, and the control of malaria by quinine, to the successful use of agents such as the sulfonamides and the antibiotics. Early in this century (1904) Ehrlich and Shiga [40] showed that an acutely fatal general infection in mice caused by trypanosomes could be cured by a single dose of a synthetic dye stuff, trypan-red. Apart from the action of the quinine in malarial infections, nothing analogous was ever shown The fact that an organic compound prepared in the laboratory was capable of curing a protozoal infection opened anew the prospect of attacking bacterial infections by similar means. Another incentive was that serotherapy, as exemplified by Behring's successful (antitoxin) treatment in diphtheria, failed to provide a universal method of treating bacterial diseases. The aniline dyes were examined intensively, and Churchman [26] in this country directed attention to the powerful action of gentian (crystal) violet. The first striking result was obtained with optochin, an alkaloid of the quinine group. Morgenroth and associates [41]

recorded that its administration cured mice whose blood was infected with virulent pneumococci, which in untreated animals caused a rapidly fatal septicemia. This achievement also was unique, because in no other animals and with no other drugs or organisms had cures been effected with similar certainty. The situation remained largely thus, except for the introduction of additional biologic agents such as antisera, antitoxins, and bacteriophages. In 1935, came the epochal discovery of the control by azo dyes of hemolytic streptococcal sepsis not only in mice but in human beings as well.[27] It was discovered afterward that sulfanilamide was the active principle in these dyes.

1. *Sulfonamides*

No other chemotherapeutic agent affected so many lives so soon after its discovery as did sulfanilamide and the related sulfonamide compounds.[42] A new era of chemotherapy for bacterial diseases has been opened. This not only includes investigations limited to practical therapeutic problems but also has led to a fundamental advance in the field of antisepsis itself in that the sulfonamides have stimulated a more rational approach in the search for new agents. The sulfa drugs make a refined and specialized attack on a specific functional unit of the bacterium in contrast with the more generalized chemical activity of the types of antiseptics previously described.

Several characteristics of the sulfonamide drugs have contributed to the rapidly increasing knowledge concerning them.[43] (1) chemical simplicity and ease of synthesis, which have permitted rapid development of inexpensive manufacture and widespread use, (2) the possibility of synthesis of a wide variety of derivatives of the original sulfanilamide; (3) effectiveness against susceptible organisms without the aid of the body's natural defenses, which permits in-vitro study, (4) dissociation as acids; and (5) the possession of a rapidly diazotized amino group, which permits accurate and simple colorimetric determinations of concentrations of the drugs in body fluids at therapeutic levels. There is detailed information as to their valuable therapeutic properties, as well as the limitations imposed on their use by toxic effects, and their failure to act on certain species of organisms. There is, however, much less certainty as to their mode of action. They are assuredly not very powerful antiseptics in the general sense of the term. For the optimal demonstration of their antiseptic or bacteriostatic properties *in vitro*, strictly defined conditions are required—specifically, the use of small inocula and specially adjusted media. A most striking discovery was that of Woods[44] and Fildes[45] who found that para-aminobenzoic acid neutralizes the sulfonamide effect on hemolytic streptococci *in vitro* as well as *in vivo*. This phenomenon has led to the conclusion that sulfonamides exert their

action by displacing an essential metabolite, apparently para-aminobenzoic acid, from a bacterial enzyme. Sulfonamides, therefore, act by combining with the bacteria, and hindering the access of foodstuffs, so that the organisms perish from starvation. This phenomenon is of great biological interest as it constitutes one of the first successful therapeutic attempts to achieve antisepsis by making a refined and specialized attack on a specific functional unit of a bacterium.

In addition to the direct or primary effect of sulfonamides on certain bacteria, a secondary factor, namely, the host effect, has been observed, and may play a part in ridding the infected individual of invading bacteria. Thus, in hemolytic streptococcus septicemias, as far as the individual is concerned, a fulminating and deadly infection has been converted by sulfonamide administration into one of extreme mildness and protraction. Phagocytosis plays an important role in gradually ridding the body of the infecting organisms.

From the standpoint of the surgeon, it is emphasized that substances other than para-aminobenzoic acid act as sulfonamide inhibitors. Thus, many local anesthetics (procaine and others) are esters of para-aminobenzoic acid and hence break down into the parent substance when injected into the tissues. Peptones, pus, and necrotic tissue have also been demonstrated to possess antisulfonamide properties all of which tend to neutralize hoped-for benefits to be obtained from usage of the drugs. The clinical application of the sulfonamides, in surgical infection, and the results thereof will be covered in Chapter XIV.

The bacteriostatic action of sulfonamides cannot involve an enzyme system that is universally present and susceptible of inactivation in bacteria, because these drugs are effective against only a limited number of Gram-positive and Gram-negative organisms. Furthermore, failure of the sulfonamide drugs to control the infection in a proportion of cases of pneumonia, wounds, burns, meningitis, gonorrhea, and so on, has been a conspicuous feature. Of special importance are those refractory cases that do not differ obviously in other respects from the usual run of responsive ones either in the species of causal organism or in clinical features. Failure of therapeutic response has been explained widely by analogy as a result of drug resistance of the organisms. The essence of this type of resistance is that the infection at first responds to the drug but the organisms become resistant before the infection is controlled. This phenomenon has been observed with other antiseptics and is easily reproduced in the laboratory. The essential features are as follows: The organisms are incubated with a chemotherapeutic agent with doses insufficient to kill them. After such treatment, the organisms become resistant to the drug. Resistant bacteria propagate resistant bacteria. Similarly, in animals if the infection is transmitted in the presence of a drug in doses

too small to affect an immediate cure, the infection finally becomes resistant to the drug. It is apparent that these organisms undergo a biological change in these instances because such drug resistance tends to persist obstinately. Another feature of this type of resistance is that the bacteria retain their virulence. Differences in the degree of the host's defensive responses undoubtedly play a part, but unfortunately we have no means of readily measuring these defensive responses. Nevertheless, the effectiveness *in vitro* of the sulfonamides has made it possible to place the responsibility for failure of therapy directly on the organisms and not the host. A simple method is available for testing *in vitro* response to sulfonamides of each patient's strain of organisms.[16] This method permits accurate prognosis of the results to be expected from sulfonamide therapy and minimizes the time wasted before recognizing the desirability of other forms of therapy, for example, the use of penicillin in sulfonamide-resistant cases. As the variety of chemotherapeutic agents available becomes larger, this *in-vitro* testing undoubtedly will have increasing application, especially in fulminating infections, where the saving of a life may depend on early use of an effective drug.

2. *Substances of Biologic Origin*

During the past few years, great emphasis has been placed on bacteria and fungi as sources of chemotherapeutic agents. It must be kept in mind that these substances are widely distributed in the plant and animal kingdom. Among plant products are quinine, emetine, chaulmoogra oil, tannic acid, Balsam of Peru, Tr. Benzoin Comp, and the recently introduced Chlorophyll and 'Phytonicides' (derived from onions, green moss), and others. From animals, we obtain toxins, antitoxins, and immune sera. The value of diphtheria and tetanus immunization by antitoxins and toxoids is established, but their value in the prophylaxis of gas gangrene is less clear. In this era of chemotherapy, the place of a specific antibody in the prophylaxis and treatment of bacterial infections must not be lost sight of, particularly where the bacteria concerned are actively toxigenic, as, for example, in infections of the *Clostridium tetani* and the gas-gangrene group where the sulfonamides and penicillin probably have little if any direct neutralizing effect on toxin. Other groups of substances of plant and animal origin, the enzymes and the flavoproteins, possess interesting properties. The enzymes may be of great significance in surgery if successfully applied to wounds to aid in the removal of slough and necrotic tissue, which are the most important inhibitors of antiseptics. Here must be mentioned also the bacteriophages, which before the introduction of the sulfonamides and penicillin enjoyed popularity, principally in the treatment of *Escherichia coli, Bacillus proteus,* bacillary dysentery, and staphylococcus infections. It is possible that the capabilities of

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action by displacing an essential metabolite, apparently para-aminobenzoic acid, from a bacterial enzyme. Sulfonamides, therefore, act by combining with the bacteria, and hindering the access of foodstuffs, so that the organisms perish from starvation. This phenomenon is of great biological interest as it constitutes one of the first successful therapeutic attempts to achieve antisepsis by making a refined and specialized attack on a specific functional unit of a bacterium.

In addition to the direct or primary effect of sulfonamides on certain bacteria, a secondary factor, namely, the host effect, has been observed, and may play a part in ridding the infected individual of invading bacteria. Thus, in hemolytic streptococcus septicemias, as far as the individual is concerned, a fulminating and deadly infection has been converted by sulfonamide administration into one of extreme mildness and protraction. Phagocytosis plays an important role in gradually ridding the body of the infecting organisms.

From the standpoint of the surgeon, it is emphasized that substances other than para-aminobenzoic acid act as sulfonamide inhibitors. Thus, many local anesthetics (procaine and others) are esters of para-aminobenzoic acid and hence break down into the parent substance when injected into the tissues. Peptones, pus, and necrotic tissue have also been demonstrated to possess antisulfonamide properties all of which tend to neutralize hoped-for benefits to be obtained from usage of the drugs. The clinical application of the sulfonamides, in surgical infection, and the results thereof will be covered in Chapter XIV.

The bacteriostatic action of sulfonamides cannot involve an enzyme system that is universally present and susceptible of inactivation in bacteria, because these drugs are effective against only a limited number of Gram-positive and Gram-negative organisms. Furthermore, failure of the sulfonamide drugs to control the infection in a proportion of cases of pneumonia, wounds, burns, meningitis, gonorrhea, and so on, has been a conspicuous feature. Of special importance are those refractory cases that do not differ obviously in other respects from the usual run of responsive ones either in the species of causal organism or in clinical features. Failure of therapeutic response has been explained widely by analogy as a result of drug resistance of the organisms. The essence of this type of resistance is that the infection at first responds to the drug but the organisms become resistant before the infection is controlled. This phenomenon has been observed with other antiseptics and is easily reproduced in the laboratory. The essential features are as follows: The organisms are incubated with a chemotherapeutic agent with doses insufficient to kill them. After such treatment, the organisms become resistant to the drug. Resistant bacteria propagate resistant bacteria. Similarly, in animals if the infection is transmitted in the presence of a drug in doses

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bacteriophage have not yet been sufficiently investigated and exploited.

Waksman[47] lists seven groups of microbial products that may be produced from an astonishingly large variety of unrelated micro-organisms These include polypeptides, such as tyrothricin, pigments, such as pyocyanin (a-oxyphenazine), lipoids, such as pyocyanase, organic bases, such as streptothricin, simple organic molecules, such as hydrogen peroxide, and others of more complicated composition, such as penicillin. It must be reiterated here that as with synthetic chemical substances, most antibiotics behave as poisons in the presence of animal tissues. Of the many antibiotics isolated to date, only five are used, or show promise of any use in medicine. These are tyrothricin, penicillin, streptothricin, streptomycin, and bacitracin. Of these, the first two are now approved by the N.N.R. for clinical application. All of these have demonstrated their ability to control or greatly inhibit the activity of bacteria in local infections. Penicillin, streptomycin, and bacitracin are the only ones that can be used systemically. Penicillin administration is entirely without evidence of toxicity, except for rare cases of sensitivity or idiosyncrasy. Streptomycin and bacitracin in their present form produce evidences of toxicity, which limit the dosage and the duration of treatment by these agents But with further improvement in their manufacture, these toxic factors may be eliminated.

A. TYROTHRICIN

This substance is an extract, first isolated by Dubos[48, 49] in 1931, from cultures of *Bacillus brevis*, a Gram-positive aerobic spore-forming soil organism. Dubos should be credited with the revival of interest in antibiotics in the United States, and it is possible that his work may have stimulated Florey, Chain, and others to exploit Fleming's[50] discovery of penicillin in 1929. Tyrothricin possesses antibacterial action against several species of Gram-positive organisms including pneumococci, streptococci, and staphylococci. (Gonococci and meningococci are also somewhat susceptible.) It consists of at least two substances, gramicidin and tyrocidin, the former being by far the more active component. Gramicidin is hemolytic although it is relatively nontoxic for other tissue cells. Its action on bacteria appears to consist at least in part, of inhibiting enzymatic action, thereby retarding growth and causing lysis of the bacteria against which it is effective. It is not much affected by body fluids such as saliva, urine, and serum. Gram-negative bacilli are extremely active inhibitors of tyrothricin; in fact, in mixed infections they may protect the otherwise susceptible Gram-positive organisms against its effect.

Tyrothricin is a protein (polypeptide) and a hemolytic agent. It is not only ineffective but even dangerous if given intravenously, and it is ineffective when administered orally. It is of value, however, for infections caused by Gram-positive organisms with which it can come in direct

is remarkably lacking in toxicity. It affects neither growth nor metabolism of animal tissues despite massive administration of many times total daily therapeutic doses. In fact, it is the least toxic of presently known antibacterial substances. The remarks made on 'resistant infections' concerning resistant strains and acquired resistance with respect to the sulfonamides apply also to penicillin, although as yet there are fewer reports regarding this than the sulfa drugs.

Penicillin has great advantages over the sulfonamides in that: (1) it is the least toxic of all known antibacterial drugs and there is no danger of the occurrence of toxic effects such as urolithiasis, nausea, vomiting, or action on blood-forming organs; (2) it is not inhibited locally by blood, pus, peptones, or necrotic tissue products; and (3) when used locally, it minimizes rather than increases exudate. Its disadvantages include: (1) specificity of action; (2) inactivation or inhibition by Gram-negative bacilli, or impaired efficiency in their presence; (3) instability; and (4) *rapid absorption after application with rapid excretion*, which necessitates frequent dosage. Oral administration, in a form that will prevent the inactivating effect of gastric secretions, has been moderately successful,[63] but the uncertainty of adequate blood levels makes this inadvisable for the treatment of serious infections.

Penicillin may be administered systemically or locally (topical, intrathecal, intrathoracic, and so on). Systemic dosage for common infections approximates 100,000 to 200,000 or more units daily, a fractional part of this being given every two to three hours. Solutions or ointments yielding 500 units per gram are suggested for topical use. For intrathecal usage, daily instillations of 10,000 to 20,000 units in 10 cc. of isotonic saline is the average dosage. For intrathoracic instillation, the dosage and volume are doubled or trebled, depending on the circumstances. Application of cold packs to the site of intramuscular injection slows adsorption. The use of penicillin as a powder together with dry plasma,[64] or of the drug *in an ointment base such as carbowax or purified lanolin*, releases it slowly on topical application.[65] Its clinical application and the results thereof will be covered in Chapter XIV.

The specificity of penicillin and tyrothricin principally for Gram-positive bacteria has prompted a search for antibiotic agents having a more over-all activity. Several agents have been discovered that are active against a variety of bacteria irrespective of their Gram reaction, among them, claviformin, clavacin, pyocyanin, penicillin acid, fumigatin, streptothricin, streptomycin, and bacitracin. The last three of these are the only ones that seem to possess properties making them promising as antibiotics for clinical use.

C. STREPTOTHRICIN

The discovery of streptothricin by *Actinomyces lavendulae* and the definition of some of its antibacterial properties were published in 1940-41 and suggest that this agent offers many possibilities as a chemotherapeutic agent, especially in the treatment of Gram-negative bacterial infections. Certain fungi and organisms of the colon-typhoid and Salmonella groups appear to be sensitive to its action, whereas others, such as the Proteus and Pseudomonas groups, show considerable resistance. Gram-positive forms are also sensitive to the action of streptothricin, but not nearly to the same degree as Gram-negative forms. Gram-positive anaerobic pathogens are relatively unaffected. It is not active against the virus of epidemic influenza. The potency of streptothricin is measured by the degree of inhibition of a special strain of *E. coli* in nutrient broth, and is expressed in *E. coli* units. The activity of streptothricin is not influenced by blood, serum, peptone, or vitamins of the B complex. It is more active in infections in experimental animals when given parenterally than when administered by mouth. It influences gastrointestinal bacteria when given orally, indicating it is not destroyed by gastric acidity. These results to date suggest that the purified product may be useful in the local and topical treatment of infected wounds and burns, as well as in those infections produced by dysentery and Salmonella organisms, but toxicity precludes its extensive systemic use

D. STREPTOMYCIN

This antibiotic was isolated in 1942 from two strains of actinomyces related to an organism described as *Actinomyces griseus*.[56] This substance resembles streptothricin in its solubility in water and selective activity against Gram-negative bacteria, and it is decidedly less toxic. Streptomycin in vitro and in experimental mice infections is active against a variety of Gram-negative and Gram-positive bacteria, but not against fungi[57-8] (fungi are affected to certain degrees by streptothricin). Thus the Gram-negative Eberthella, Salmonella, Escherichia, Shigella, Klebsiella, Brucella, Pasteurella, and Proteus groups are sensitive to streptomycin.[59-60] In contrast, some strains of the Pseudomonas group show considerable resistance. Staphylococci, hemolytic streptococci, and pneumococci likewise are sensitive, whereas all members of the spore-bearing, anaerobic group are highly resistant. Experimental and clinical tuberculosis are favorably altered.[61] Streptomycin is most effective when given parenterally, although adequate doses by mouth sterilize the gastrointestinal tract of lactose-fermenting organisms. Streptomycin is rapidly excreted by the kidneys after systemic administration, and must be administered by frequent injections in order to maintain an effective blood level, in doses of 0.5 g. every three or four hours (see Ch. XIV).

E. BACITRACIN

The most recent addition to this group of clinically effective and relatively nontoxic antibiotics is Bacitracin, discovered in 1943 and reported first in 1945.[62] It is produced by a spore-forming bacillus of the *Subtilis* group. It has a wide range of antibacterial activity, which, however, runs roughly parallel with that of penicillin. Its chief advantage over penicillin is that since it is not inhibited by the Gram-negative rods and other organisms that produce penicillinase, it is often active against infections resistant to penicillin. It is more slowly absorbed and more slowly excreted from the body, so that it may be given at greater intervals. Its clinical results will be more fully described in Chapter XIV.

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CHAPTER VI

Infection in Clean Operative Wounds

ABOUT HALF the operations performed on a *general surgical service are in fields that are clean* and relatively free from bacteria. Such clean fields are on any part of the external body surface at a sufficient distance from the orifices of the body such as the nostrils, mouth, vagina, urethra, and anus, and away from the internal mucous-membrane-lined surfaces so that wounds made at operation are not contaminated by the organisms normally present around such orifices and on such mucous-membrane-coated surfaces. This clean group offers an opportunity to check up on the sterile technic of the operating room and the operating technic of the surgical staff. Although certain operating fields are clean, it is generally recognized that every wound that is made in the operating room becomes contaminated with bacteria during the course of the operation.

Whether or not infection develops in such an operative wound depends upon the number and virulence of the contaminating bacteria and the *local and general defenses of the body against infection.* The sources of bacterial contamination have been mentioned in Chapter III on sterile technic, and procedures have been outlined for minimizing or preventing contamination.

The success of the efforts made to minimize this contamination of the wound can be measured only by an accurate count of the number of infections that develop in these cases. It cannot be a matter of impressions but an actual count carried out according to a *definite plan* by someone especially assigned to the study for a fairly long period of time.

That impressions are completely unreliable was clearly demonstrated by the staff of the Presbyterian Hospital, New York, in 1925, when it was thought that the incidence of infection in clean operative wounds was about 2 per cent, whereas an actual count revealed it to be 13.6 per cent. This discovery shocked the members of the surgical staff to such an extent that a study was inaugurated that was carried on continuously for 18 years and was discontinued only when the shortage of residents and the extra burden of work incident to the war made it impossible to *continue. The author of this book conducted the study* and personally reviewed and analyzed all of the records during the first 10 years and

advised and directed the surgical residents who continued the study during the last 8 years. The purpose of the study was not only to find out the incidence of wound infection but also to discover, if possible, the causes of the infections and the sources of contamination, to minimize those causes, to close the door to those sources of contamination, and to keep the whole operating staff on the alert to maintain the highest standards of sterile technic and the gentlest kind of operative trauma. It has made the staff bacteriology-minded and it has developed a friendly rivalry to see who can have the fewest infections or be kept entirely off the infection list from year to year.

I. MASKING AS RELATED TO OPERATIVE-WOUND INFECTIONS

It is regrettable that it required the catastrophe of an epidemic of wound infections to arouse the staff to make an investigation. We should have maintained a continuous check on the results of our operating-room technic from the time of its inauguration. Many of the details of the sterile technic had been passed down to us by hospital tradition or by word of mouth from our immediate predecessors on the staff. Most of these details we had taken for granted without questioning their rationale. We assumed that at some time in the past the reason for this or that step in our procedure had been adequately demonstrated. We thought that we were getting satisfactory results in our wound healing. Our study, however, clearly demonstrated that our results left much to be desired. We soon found a very glaring weakness in the technic, which opened a wide door for bacterial contamination of the wound. The 'epidemic' of suppuration consisted in a series of eight hemolytic streptococcal wound infections in the winter and early spring of 1925. Our immediate concern was to find the source of these hemolytic streptococci. We cultured every kind of material that had been prepared for the sterile field—those that had been boiled, those that had been autoclaved, and those that had been soaked in antiseptic—but found no trace of the hemolytic streptococcus. The sterile-water tanks occasionally contained Gram-negative organisms but never a hemolytic streptococcus. The exposure of blood-agar plates to the air of the operating rooms for several hours on many occasions yielded only one colony of hemolytic streptococcus. Cultures from the skin of the patient seldom gave this organism. It was only when we came to examine the hands and the noses and throats of the operating personnel that we found a field of activity for the hemolytic streptococcus. Much to our surprise, 33 per cent of the operating-room staff harbored this organism in the throat, and many individuals carried it in the nose as well. The organism could also be

recovered occasionally from the hands of the doctors. It was evidently picked up from the dressings and bedclothes of patients suffering from hemolytic streptococcal lesions and may have been transferred by the doctor to his mouth. Cultures from the hands of the doctors just before dressing these cases seldom yielded these bacteria, but directly afterward they were almost invariably present.

The above findings suggested the direction for our immediate researches. A survey of noses and throats of the entire operating personnel was made. Fortunately (and I use the word advisedly for I realize how lucky we were), just at that stage of our study, a clean hernia developed a hemolytic streptococcal wound infection. When we looked up the record, we found that 3 members of the operating team were carriers of the hemolytic streptococcus. It had been cultured from the throats of 2 of the doctors and from the nose and throat of the instrument nurse, just a few days before the operation. By a strange coincidence also, as it proved, cultures of the patient's nose and throat yielded hemolytic streptococci. The question then arose: Which of the extraneous strains matched the organism that had been recovered from the wound? By the injection of rabbits with the several strains, agglutinating sera were obtained and then by agglutination and cross-absorption of agglutinin tests, it was indisputably shown that the organism from the wound was identical with the organisms from the nose and throat of the instrument nurse, and entirely different from the strains from the patient's nose and throat, and the strains from the 2 doctors. The evidence was overwhelming that the organism had been discharged upon the sterile field from the unmasked nose or throat of the instrument nurse. Thereupon, the order was given for all operating teams, without exception, to mask both nose and mouth with fine-meshed, four-ply gauze masks. This measure, we found, reduced the number of organisms deposited upon a blood-agar plate held in front of a person so masked to approximately the number deposited on a control plate some distance away. This complete masking was later extended to all persons, both sterile and unsterile, entering the operating room, except the patient himself. Thereupon, our hemolytic streptococcus wound infections were immediately reduced to casual numbers that could probably be accounted for by contamination from other sources.[1-3] The anesthetists objected to masking, because they said it was difficult to breathe when the fumes of the anesthetic were added to the annoyance of the masks, but when they were exempt for a few months, 3 cases of hemolytic streptococcus infection developed following thyroid operations, and the proximity of the unmasked anesthetists in these operations rendered them suspicious sources of these organisms. After that there were *no exceptions*. Also, if any member of

The last two methods, shown in Tables I and II, are recommended to the chiefs of all surgical staffs for use in similar studies. Punch cards may be analyzed by machine (International Business Machine Co.) or by hand (McBee System). For large numbers of cases, the punch cards are preferable. For small numbers, the record cards are satisfactory. A continuous study by some responsible member of the staff will yield dividends in continued interest on the part of the staff in sterile technic, in prompt lowering of the incidence and severity of infections, in shortened periods of hospitalization, and in more satisfied and happier *patients.*

TABLE I

WOUND HEALING				
Operations	1st	2nd	3rd	
Clean				Name
Hematoma				
Stitch abscess				Operation
Necrosis				
Disruption				Attending
Drained				
Silk				Operator
Catgut				1st Asst.
Trivial infection				2nd Asst.
Serious infection				Sterile nurses
Analysis				*Remarks*

In presenting in Table III the results of our 18-year study, we should point out that the definition of the words 'wound infection' is 'the

reaction in or around the wound due to the activity of bacteria or their products.' An infection is serious if there are systemic manifestations of inflammation, if it progressively destroys tissue, or if it unduly prolongs hospitalization. It is trivial if there are only local signs of inflammation, if it is not progressive, and if it does not prolong hospitalization.

TABLE II

SUTURE MATERIAL	DRAINAGE	WOUND HEALING
0 None	0 None	0 No complications
1 Silk	1 Cigarette	1 Infect.—trivial
2 Catgut	2 Rubber tube	2 stitch abscess only
3 Silver	3 Penrose	3 serious
4 Steel	4 Tampon	4 Skin separation
5 Dermol for skin	5 Packing	5 Hematoma
Y Other—specify	6 Rubber dam	6 Necrosis
	Y Other—specify	7 Hemorrhage
	9 OPER. IN TREATMENT ROOM —specify	8 Disrupt. of wound without evisceration
	X BREAK IN TECHNIC—specify	9 Disrupt. of wound with evisceration
		X Fistula
		Y Other wd.-healing complic.—specify
		Z No wound healing

Table III shows a gratifying reduction in the percentage of both serious and trivial infections, even though the number of clean cases operated upon each year has steadily increased (until the last year of the study when a smaller staff on account of the war resulted in a reduction of cases). This situation reflects certain changes in our technic as a result of the study. The first change was early in 1925, when we inaugurated complete masking of nose as well as mouth following the demonstration of contamination of the hernia wound by a hemolytic streptococcus from the unmasked nose of the instrument nurse. The policy of adequate masking was adopted early enough in 1925, so that that year shares with later years in the practical diminution of hemolytic streptococcal infections. It is to be noted in the table that there was a drop both in the number of cases and in infections in 1928, when we moved from the old hospital to the new Medical Center. We believe that this drop was due in part to the fact that in the old hospital the operating room was on the street level and on a busy corner with heavy traffic. In the new hospital, the operating rooms are on the sixteenth floor with a considerable reduction of air contamination (see below).

TABLE III

SHOWING THE PERCENTAGE OF SERIOUS, TRIVIAL, AND TOTAL INFECTIONS IN CLI SURGICAL CASES OVER A PERIOD OF 18 YEARS IN THE PRESBYTERIAN HOSPITAL NEW YORK CITY

YEAR	CLEAN CASES	PER CENT OF INFECTION		
		Serious	Trivial	Total
1925	558	3.6	10 0	13 6
1926	581	3.7	11.2	14.9
1927	653	2.5	12 5	15.0
1928	640	1.6	9.2	10.8
1929	771	2 2	6 6	8.8
1930	747	3 1	6 7	9.8
1931	950	1 7	5 4	7.1
1932	1,053	1.1	4.2	5 3
1933	1,132	1.1	3.6	4.8
1934	1,279	0 9	2 8	3.7
1935	1,417	0.6	2 5	3 1
1936	1,558	0 6	2.5	3 1
1937	1,614	0 8	1 4	2 2
1938	1,668	0 5	1.6	2.1
1939	1,725	0 6	2 0	2 6
1940	1,852	0 6	1 5	2 1
1941	2,039	0.7	1 8	2 5
1942	1,868	0.8	1 8	2 6

III. MINIMIZING AIR CONTAMINATION

Lister[7] believed that organisms dropping from the air played a important role in wound infection, and developed his elaborate appara tus for spraying the atmosphere. It was later believed that contamina tion from the air was one of the relatively minor factors. However, wit the other sources greatly reduced or eliminated, if nothing is done t minimize contamination from this source, it becomes relatively importan again. If a blood-agar plate is exposed to the air of an operating roon for an hour and then incubated, its surface will be more or less thickl covered with dust particles, and beneath some of these particles, colonie of bacteria of various kinds develop. Staphylococci will make up th majority of these colonies. Yellow and white, hemolytic and nonhemolyti will all be represented. *Bacillus subtilis, Escherichia coli,* nonhemolytic streptococci, and diphtheroid bacilli will be present in smaller numbers Hemolytic streptococci will appear rarely. Yeasts and molds are rela-

tively common. The number of colonies will depend upon a number of factors. In the old Presbyterian Hospital in New York, cultures from the ground-floor operating room were consistently more numerous than those from the upper floor and yielded roughly two colonies for every minute of exposure. In the new hospital, in the Columbia Presbyterian Medical Center, with the operating rooms on the sixteenth floor, the colonies were found to be just half as numerous, namely, one colony per minute of exposure. The fresh air supplied to these rooms is filtered. But this filtration is nullified by the opening of doors, the occasional coming and going of assistants, the spread of blankets, and the general movement of the people connected with the operation. In these rooms, visitors are required to observe the operations from a balcony protected by plate glass. If visitors are permitted to come on the floor of the operating room, as is the case in many places, it is obvious that the number of air organisms are materially increased, especially when the visitors are not properly masked. The area of a blood-agar plate is approximately seven square inches. The sterile field including the operating table and instrument tables might vary from 4000 to 7000 square inches. It would seem fair to assume, therefore, that in a busy operating room in the course of an hour from 35,000 to 60,000 bacteria fall upon the sterile field It is obvious that these figures may be materially reduced by minimizing the activity within the room and the opening and closing of doors, by removing objects that collect dust, and by refusing admittance to unnecessary persons. Later we further cut down air contamination by covering the sterile tables with canopies, and still later by ultraviolet radiation, as described in Chapter IV [8-10] (see Table XIII).

Table III shows another drop after 1930, with a sudden fall in 1931, and a more gradual decline thereafter. This drop, we are confident, was due chiefly to the change from catgut to silk for ligature and suture material. The drop in the infection incidence in thyroid cases in 1930 was offset by an unusual incidence of infection in the fracture cases for that year.

IV. SILK VERSUS CATGUT AS LIGATURE AND SUTURE MATERIAL

During the first five years of our study it was evident that certain types of operation regularly had a higher incidence of infection than others. Radical mastectomy, partial thyroidectomy, recurrent hernia, ventral hernia, open reduction of fractures headed the list. There seemed to be a reason for this in the nature of the operation, except in the case of partial thyroidectomy. Practically all of the thyroid operations were being done by the two surgeons associated with the thyroid clinic, and

TABLE IV

THYROID OPERATIONS

	TOTALS	INFECT.	PER CENT	HEMAT.	PER CENT
1926	57	10	18	16	28
1927	77	13	17	30	39
1928	78	8	10	29	37
1929	163	20	12	60	38
1930	189	4	2	29	15
1930—Catgut	35	2	6	15	43
1930—Silk	154	2	1	14	9
1931	216	4	2	29	13

1930—Average number days in hospital catgut cases	12
1930—Average number days in hospital silk cases	9

TABLE V

1931—SILK VERSUS CATGUT

	INGUINAL HERNIA					
	Total	Hem	Per cent	Ser	Triv.	Per cent Inf.
Catgut	100	14	14	1	4	5
Silk	49	0	0	0	0	0
Silk and catgut	8	2	25	0	3	38
Not stated	5	0	0	0	0	0
Totals	162	16	10	1	7	5

	FRACTURE SERVICE					
Catgut	38	11	29	2	5	18
Silk	79	1	1	0	0	0
Silk and catgut	10	4	40	0	1	10
Not stated	10	0	0	0	0	0
Totals	137	16	12	2	6	6

Table VI

1932—Silk Versus Catgut

	Silk					Catgut				
	Total	Hem	Per cent	Inf	Per cent	Total	Hem	Per cent	Inf	Per cent
All cases	656	30	4.6	15	2.3	306	20	6.5	28	9.2
Inguinal hernia	113	4	3.5	3	2.6	36	4	11	2	5.6
Ventral hernia	13	0	0	0	0	13	0	0	4	30.0
Rad. mastectomy	6	1	17.0	1	17.0	7	1	14	2	28.0
	Both					**Not stated**				
All cases	19	0	0	2	10.5	72	8	11	11	15
Inguinal hernia	8	0	0	2	25	1	0	0	0	0
Ventral hernia	0	0	0	0	0	0	0	0	0	0
Rad. mastectomy	0	0	0	0	0	7	5	71	4	57

it did not seem possible for them to reduce their high incidence of hematomas and infections. In the summer of 1929, while one of the thyroid surgeons was on his vacation, the author had the opportunity of performing 5 thyroid operations in close succession. As had been his habit since his experience in thyroid surgery in Peking, China, under the leadership of Dr. Adrian S. Taylor, a former pupil of Dr. William Halsted, the author used fine silk entirely for ligature and suture material in these cases. The differences in the healing of these wounds compared with the catgut-sutured wounds was obvious to all who saw them The second thyroid surgeon observed these results and was persuaded to try silk. He performed 10 consecutive cases with silk, all of which healed promptly and kindly without trace of hematoma or infection. He then sutured 10 cases with catgut and obtained 4 hematomas and 2 infections. He was convinced of the superiority of silk and determined to use it entirely thereafter. Some months later, the other thyroid surgeon was also persuaded to make the change. The results obtained were both surprising and most convincing to him. These are shown in Table IV.

When the report of the year 1930 was presented to the staff, it was suggested that the use of silk be extended further, particularly to those cases yielding such a high percentage of infection, namely, open reduction of fractures, recurrent hernias, and radical mastectomies. The

TABLE VII

1933—WOUND INFECTIONS IN RELATION TO SUTURE MATERIAL

ALL CASES

	Total	Clean	Per cent	Ser	Per cent	Triv	Per cent	Tot Inf	Per cent	Hem.	Per cent
Silk	526	511	97	3	0 6	12	2 0	15	3	20	4 0
Catgut	351	322	92	6	2 0	23	7 0	29	8	27	8 0
Linen	138	132	96	3	2 0	3	2 0	6	4	12	10 0
Combined	13	12	92	0	0	1	8 0	1	8	1	8 0
Not stated	104	101	97	1	1 0	2	2 0	3	3	2	2 0
Total	1,132	1,078	95	13	1 1	41	3 6	54	5	62	5 5

THYROIDS

	Total	Clean	Per cent	Ser	Per cent	Triv.	Per cent	Tot Inf	Per cent	Hem	Per cent
Silk	107	107	100	0	0	0	0	0	0	12	11
Catgut	0	0	0	0	0	0	0	0	0	0	0
Linen	129	123	95	3	2	3	2	6	5	12	9
Combined	0	0	0	0	0	0	0	0	0	0	0
Not stated	11	11	100	0	0	0	0	0	0	0	0
Total	247	241	98	3	1	3	1	6	2	24	10

PRIMARY INGUINAL HERNIAS

	Total	Clean	Per cent	Ser	Per cent	Triv	Per cent	Tot Inf	Per cent	Hem.	Per cent
Silk	141	137	97	1	0 7	3	2	4	3	3	1
Catgut	40	38	95	2	5 0	0	0	2	5	3	13
Linen	4	4	100	0	0	0	0	0	0	0	0
Not stated	3	3	100	0	0	0	0	0	0	0	0
Total	188	182	97	3	2.0	3	2	6	3	5	3

RADICAL BREASTS

	Total	Clean	Per cent	Ser	Per cent	Triv.	Per cent	Tot Inf	Per cent	Hem	Per cent
Silk	9	9	100	0	0	0	0	0	0	0	0
Catgut	10	6	60	0	0	4	40	4	40	2	20
Linen	0	0	0	0	0	0	0	0	0	0	0
Not stated	9	7	78	0	0	2	22	2	22	1	11
Total	28	22	79	0	0	6	21	6	21	3	11

fracture-service surgeons decided to use it for all of their clean cases, and a certain group of the general surgeons agreed to use it on all hernias. In the next year's report, an opportunity was given to compare silk and catgut in these two large groups. Again the superiority of silk was demonstrated unmistakably, as Table v shows clearly.

Table VIII

A Comparison of Infection Rates Following the Use of Silk and Catgut in Clean Operations over an Eleven-year Period

Year	Silk		Catgut	
	Total	% Infected	Total	% Infected
1932	656	2.3	306	9.2
1933	526	3.0	351	8.0
1934	864	2.0	316	7.0
1935	991	2.7	423	4.0
1936	1,149	2.7	407	4.4
1937	1,296	1.0	318	4.1
1938	1,297	1.7	314	3.5
1939	1,487	2.0	214	6.5
1940	1,544	1.8	258	3.9
1941	1,588	2.2	222	4.1
1942	1,391	2.7	173	3.5
Totals	12,792	2.2	3,302	5.4

During the next 2 years, silk was substituted more and more for catgut. In 1932, we were able to compare silk and catgut in three large groups (see Table vi).

In 1933, a large majority of all clean wounds were sutured with silk. For the first time, we were able to compare all of the cases closed with silk with all of the cases closed with catgut (see Table vii). Whipple[11] then reported his conversion to silk.

In 1933, the two thyroid surgeons experimented with linen as a substitute for silk, but their results were unsatisfactory, as Table vii so clearly shows.

In the eleven years from 1932 to 1942 inclusive, a comparison was made of the percentage of infections, comparing the silk with the catgut cases, with ever-convincing proof that the use of silk was superior. This is shown in Table viii—the consistency of the results as well as the total figures make these percentage differences statistically significant. It is of

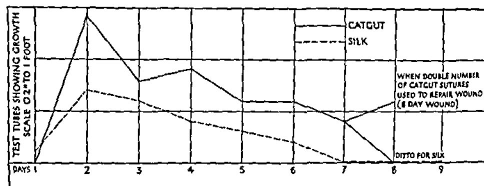


FIG. 1 Silk and catgut sutures comparisons (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

interest also that the number of clean cases operated on with catgut steadily declined, while the silk cases rapidly increased, indicating how the study gave a rational basis for a modification of operative technic.

Halsted,[12] following the lead of Kocher in Berne, substituted silk for catgut in suturing clean cases, partly because of the uncertainty of catgut sterilization in those days, and partly because he noticed that the wounds healed 'more kindly' even when there was no infection. There was less redness, swelling, and induration. Thus, he became the chief advocate of the use of silk in this country, and his pupils have almost all followed his example and spread this gospel abroad. On the other hand, few surgeons who did not come under his influence either directly or through his followers and pupils have been convinced that he was eminently right in his conviction nor have they been willing to change their technic in this respect.

It seems strange that Halsted did not attempt to prove his point either by well-controlled clinical or careful laboratory experiments. He was sure of his ground on the basis of his own experience and his own observations. In 1925, Goff [13] made a study of wound infections in clean cases and presented fairly good evidence that wounds sutured with silk were less likely to become infected than wounds on which other materials were used. Our results amply confirm the impression that he obtained from his study. In 1945 Vivier,[14] one of the senior fellows in our surgical department, at the suggestion and under the supervision of the director of the service, added clean-cut evidence from a series of experiments with animals, which strongly supports the view that silk is superior to catgut. With rigid operating-room technic, he produced two wounds extending through the wall of the abdomen in a large series of rabbits. One side was sutured in layers with finest silk and the other with the finest catgut. Each side was operated on with completely separate sterile set-ups and took the same length of time. One animal in the series was

sacrificed each day for ten days. Cultures were made from these wounds in a dustproof chamber, and by taking approximately one cubic centimeter of tissue from each side, an effort was made to estimate the number of viable bacteria actually present on its surface by shaking the tissue in five cubic centimeters of broth and diluting this down through a series of one to five dilutions. In practically every case, the catgut wounds contained more bacteria than the silk wounds, as Figure 1 shows. Furthermore, microscopic sections showed that the cellular and fluid exudation around the strands of catgut was considerably greater in amount than around the silk. Thus there exists both clinical and experimental evidence of the superiority of silk (see Figs. 1-2, Ch. xviii)

V. CATGUT AS A SOURCE OF INFECTING ORGANISMS

Catgut has often been blamed for wound infections, and there is no doubt that it has been responsible for certain of the cases caused by anaerobic spore-forming organisms, such as the tetanus bacillus and the gas-gangrene group of bacteria. These were more common a generation ago than they are now. Welch[15] once said that these wound infections constituted one of the chief reasons why Halsted substituted silk for catgut as suture and ligature material. This point will be mentioned in more detail below. In 1923 two postoperative infections due to Vibrion septique in the Presbyterian Hospital were directly traced to inadequately sterilized catgut. The catgut firm was made liable in a court of law. In 1927 the author was asked to see a patient with a postoperative wound infection, in another hospital. The contamination was traced unmistakably to inadequately sterilized catgut sold by another firm. Death occurred in the latter case and in 4 similar cases.[16] These fatalities led to a study of the methods of catgut manufacture and its sterilization, as well as tests for its sterility. Several of the catgut firms followed the study closely and modified their methods according to its findings.[17] A standard method of testing for sterility was developed.[18] This has been accepted and is now said to be employed by most of the catgut firms. Unfortunately, neither the American College of Surgeons nor the American Medical Association was willing or financially able to set up a laboratory control for determining, by periodic tests of specimens of catgut purchased on the open market, whether or not the products then sold were measuring up to the standard of sterility. This is now being done by the Food and Drugs Administration. But the test for catgut sterility is one that can be carried out by any laboratory equipped for anaerobic bacteriology, and the proposed tests should be made by the department laboratory before any particular brand of catgut is adopted by any surgical clinic. Although improperly sterilized catgut may at times

contain the organisms of tetanus or gas gangrene, it probably very rarely contains nonspore-forming, heat-susceptible organisms, like staphylococcus and streptococcus, which are the commonest organisms of operative-wound infections.

We believe that the superiority of fine silk over catgut is due to the following facts: First of all, hemostasis is better, for the silk knots do not become untied as easily as catgut knots, particularly when the wound surfaces move more or less constantly as in the thyroid operation. Second, the cellular and fluid reaction about silk is minimal, whereas the reaction about catgut is maximal. Silk is almost inert in the tissues. Catgut is dead tissue, which must be digested. In the third place, the use of silk automatically requires the surgeon to be more gentle with the tissues. His sutures and ligatures are less likely to be pulled tightly enough to strangle tissue. This strangling of tissue often happens when catgut is used and increases with the postoperative edema. For these reasons, with a certain inevitable amount of bacterial contamination, infection is more apt to develop in tissue in which catgut has been used than where silk has been employed. Halsted observed, and our statistics as well as our tissue sections prove, that wounds sutured with both silk and catgut are more apt to become infected than when either is used alone. This is probably due to the fact that in a wound in which organisms are able to gain a foothold easily, silk acts as a foreign body, which must be extruded. For all these reasons, silk should not be used in clinics or by operators who are not willing to strain every effort to minimize contamination by all of the means mentioned above that have been devised for that purpose, or by those who are not willing to minimize trauma to the tissues or tension of the tissues during the operation, or by those who are not willing to take the time to obtain perfect hemostasis, or by those who are not willing to mask adequately both nose and mouth.

VI. DRAINS AND HEMATOMAS

During a seven-year period, we studied the role of drains in the production of infections. In every year but one, a larger percentage of infections developed in the drained cases than in the undrained, and the average percentage of infections in drained cases greatly exceeded that

TABLE IX

ASSOCIATION OF DRAINS AND HEMATOMAS WITH INFECTION—AVERAGE 7 YEARS

	Per cent
Drained, infected	11 5
Not drained, infected	7.6
Hematomas, infected	20.4

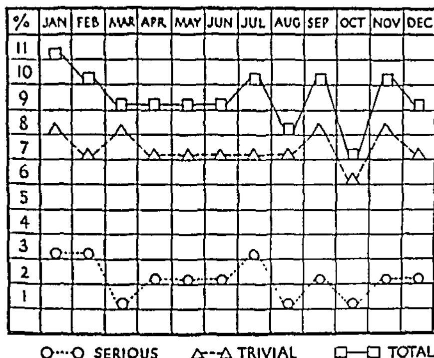


FIG. 2. Seasonal incidence of wound infection composite curve of 9 years (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

in the undrained cases, but we cannot be sure whether this was due to the drains or to the condition that seemed to the surgeon to require drainage (Table IX).

There is no question, however, about the role of hematomas. Wounds that develop collections of blood or serum are unquestionably more prone to become infected, because of organisms introduced either during the operation or later during dressings. Our studies have seemed to show that wounds may become infected following secondary contamination at the time of dressings as well as primarily from contamination introduced at the time of operation. This applies chiefly to wounds that develop hematomas or necrosis of skin from excessive tension. In a few cases each year, there has been a good excuse for the development of an infection independent of the operating-room technic. These points are brought out in Table X.

TABLE X

PROBABLE SOURCE OF INFECTION—1931

	Serious	*Trivial*
Contaminated at operation	11	34
Contaminated at dressing	2	15
(Alibi) contaminated before operation	3	2
Questionably counted	2	3

The composite curve showing the seasonal incidence of wound infection is shown in Figure 2. It does not show the spring peak that appeared in Dr. Walker's curve [6] and in our own [3] when hemolytic streptococcus was prevalent, before the period of adequate masking.

VII. THE INCIDENCE OF INFECTION IN RELATION TO THE TYPE OF OPERATION

One would expect that the site of operation would be an important factor in the incidence of infection and that certain tissues would be more susceptible than others. If we add up the score over a period of 7 years for such large groups as thyroids, hernias, celiotomies, and bone and joint operations, we find that the thyroids have a significantly low figure, whereas the others yield remarkably similar percentages (*see Table XI*).

TABLE XI

SHOWING THE INCIDENCE OF INFECTION IN THE FOUR MAJOR OPERATIVE GROUPS

YEAR	THYROIDS	% INF	HERNIAS	% INF.	CELIOTOMIES	% INF.	ARTH. & OPEN RED	% INF.
1935	271	3 3	246	1.6	221	2 7	197	1.0
1936	336	1 5	283	3 1	175	3 4	234	2.5
1937	345	0 3	328	2 7	158	1 8	222	2.2
1938	335	0 3	245	4 1	221	1.8	210	2.4
1939	294	0 3	259	2.7	234	4.3	261	2.3
1940	350	0 3	267	3 4	291	3 1	242	2.9
1941	380	0.0	289	1 7	257	1 2	281	5.0
Total	2,311	0 8	1,917	2 8	1,557	2 6	1,647	2.7

The thyroids surpass in number all other groups, and since 1935, the incidence of infection has been almost negligible. This is in sharp contrast to the first 5 years of the study when almost all of the thyroids were operated on with catgut and the infection rate was 13.7 per cent with 33.3 per cent of hematomas in spite of drainage. One might try to explain the improvement by the increasing experience of the operators, but in the early years, the thyroids were almost all done by the two surgeons associated with the thyroid clinic, whereas in the later years, as the numbers increased, more and more cases were done by the younger members of the staff. All of the operators agree that the major factor is the use of silk rather than catgut, which provides better hemostasis and calls forth less exudation of fluid in a constantly moving operative area.

This has made drainage unnecessary; the wounds have healed 'more kindly,' and the period of hospitalization has been shortened.

Although the figures in the other groups are closely similar, when they are broken down, we find considerable difference in the incidence of infection in some of the subgroups. For example, among the hernias, we regularly found more infections with the recurrent and ventral hernias than among the primary inguinal or femoral cases. Scar tissue and possibly residual organisms play important roles here. When two hernias were done at the same time, we found a higher incidence of infection than in single cases and usually it was the second side that became infected, suggesting that it received contamination from a field longer exposed to air contamination. This discovery led to a new sterile set-up for the second side, and this change in technic obviated the difficulty.

A breakdown of the fracture-service figures shows a higher incidence of infection in the open-reduction cases than in the partial ostectomies and arthrotomies. The factors of extensive tissue damage, prolonged operation, and introduction of foreign bodies all play a part here.

One group of cases not included in the four main categories but which yielded a high percentage of wound infections in the early years of the study were the radical mastectomies. Extent of damaged surface and skin tension probably play an important role in infection in radical mastectomy but postoperative edema and exudation in the operative field may add to the tension existing at the end of operation and change what appears to be a closure 'without much tension' to one with too much tension. With ventral hernia, this same tension and exudation may occur in the deeper tissues rather than in the skin. In exploratory celiotomy, the extent of and the trauma incident to the exploration should be considered as contributing factors to infection as well as the general condition of the patient in which only an exploration is done.

VIII. SUMMARY OF OPERATIVE FACTORS RESPONSIBLE FOR OPERATIVE-WOUND INFECTIONS

To sum up the factors from the point of view of the operative technic, one might say that infection depends in some degree on the type of operation, the tissues involved, the extent of tissue injury, the degree of tissue tension, the necessity for drainage, the frequency of hematomas, and the duration of the operation; and also to a large degree on the kind of suture material that is used and perhaps on the variations in technic that these materials require. We must seriously question every step in the technic of operations that year after year show a large incidence of infections. These include radical mastectomy, ventral hernia, exploratory celiotomy, excision of lipoma, and open reduction of fracture.

IX. THE BACTERIA CAUSING WOUND INFECTION

And now we come to the central causative factor, the bacterial tamination. We cannot tell what organisms get into the wound wit cultivating the whole wound at the beginning, frequently during, ar the end of the operation. This has not been done frequently enoug warrant presenting the data. Cultures have been taken of air cont nants during the operation, but these are not wholly indicative of enters the wound, which is the concentration point into which n objects go from all over the sterile field. The washing of the woun the end of operation is probably more important from the point of of removing bacterial pabulum than of removing the organisms th selves. Unquestionably, countless bacteria of all kinds contaminate o ative wounds and are never heard from again, having been destroyec the tissues. It may be that some day we shall have an agent that destroy all of the organisms present in an operative wound when operation is over without injuring the tissues, but such an agent has yet been made available. Certainly, infection cannot take place with the presence and activity of bacteria. In the study made at the Pres terian Hospital, we have contented ourselves with taking cultures if when signs of inflammation appeared. The identification of these org isms has given us a clue to their source and this knowledge has giver an opportunity to try to dry up that source and so prevent a contami tion of subsequent cases from the same source. The combined figu covering eight years of this study, which are representative of the wh period, are shown in Table XII.

During this time, contamination from the noses and throats of operating-room personnel was reduced by careful masking, and in t period we believe that the organisms came largely from other sourc

It is seen that the staphylococci comprised by far the most comm groups, occurring in two-thirds of all the infections. The hemoly *Staphylococcus aureus* was found in almost a third of all the seri infections, and the *Staphylococcus albus* was the chief offender in t trivial infections, being present in almost two-fifths of the cases. T hemolytic and nonhemolytic streptococci were found in 4 and 5 per ce respectively. The hemolytic variety was more serious than the nonhem lytic, and both of them were found more often in serious than in triv infections. The coli and proteus groups were somewhat more likely produce serious than trivial infections, and the Pseudomonas grou although not occurring so frequently, was invariably serious. On the oth hand, the subtilis group and the diphtheroid bacilli, which are ord narily not considered pathogenic, occasionally caused trivial infectio

and when found were frequently in pure culture. Among the 'others,' the only organism of importance was *Clostridium welchii*, which occurred once in an amputation stump and was probably present in the gangrenous foot requiring amputation.

Table XII

Incidence of the Various Organisms Found in Clean-wound Infections for 8 Years of Complete Masking at Operation

Organisms	Serious per cent	Trivial per cent	Total per cent
Hemolytic streptococcus	9	2	4
Nonhemolytic streptococcus	7	4	5
Hemolytic *Staphylococcus aureus*	32	16	22
Nonhemolytic *Staphylococcus aureus*	13	11	14
Staphylococcus albus	19	38	31
Coli group	7	6	6
Proteus group	3	2	3
Pseudomonas (pyocyaneus) group	4	0	1
Subtilis group	4	7	6
Diphtheroids	0	6	4
Others	2	1	4

Our studies in ultraviolet radiation have convinced us that this agent can reduce the bacterial air contamination significantly. One operating room was therefore equipped with a central unit incorporated in a Scialytic lamp, which focused the rays about a foot above the operative field. The heat of the illuminating unit caused a gentle current of air up through this cone of concentrated radiation. Six wall tubes were so arranged that the whole room had a fairly even intensity of the ultraviolet rays. A study of the results over a period of time is summarized in Table XIII.

Table XIII

Cases done under ultraviolet radiation:			
No. of cases, clean	97	Total incidence of infection	1.5%
contaminated	28		3.4%
Average colony count	(114 cases)	.1942 per plate per minute	
Control cases done without radiation:			
No. of cases, clean	1,852	Total incidence of infection	2.1%
contaminated	964		8.3%
Average colony count	(41 cases)	.9502 per plate per minute	

X. PROLONGED HOSPITALIZATION OF INFECTIONS

It seems important for surgeons, as well as hospital superintendents, to know that patients with clean wounds that become infected have to stay in the hospital about twice as long as they would if their wounds had remained clean. This fact is shown in Table xiv.

TABLE XIV

DAYS IN HOSPITAL AFTER OPERATION—AVERAGE 5 YEARS

Clean	12.4
Infected	23 3
Hematoma	16 1

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Infection in Accidental Wounds

SINCE TIME immemorial, man has been living in an environment of bacteria from which he is protected by a layer of skin on the outside and a layer of mucous membrane on the inside. It has been demonstrated that certain bacteria, principally of the staphylococcal group, can find entrance into the ducts of the sebaceous glands, multiply there, produce their poisons, destroy tissue, and enter the subcutaneous tissues Hemolytic streptococci can pass through an intact but inflamed throat. Tubercle bacilli can penetrate the intact wall of the intestine. But for the most part, the entrance of bacteria into the interior of the human body depends upon a break in the continuity of the protective covering. Such breaks occur frequently in times of peace as a result of accidents of one sort or another, but they have steadily increased as man has gradually increased the speed of his moving about. The machine age has subjected man to greater and more frequent trauma. The human body has developed no adequate safeguard to meet this trauma, and the safety devices that man has developed do not adequately protect him.

Accidents resulting from high-speed automobiles, trains, and airplanes produce injuries to the human body that differ from accidents occurring in the horse-and-buggy days, chiefly because of the increased momentum of the moving object. They may produce injury to deep tissues or be transmitted from the site of contact to distant parts of the body. The chief importance, however, of modern accidents is the greater number of people involved and, therefore, the greater variety of injuries that are incurred

All of the tissues of the body are subjected to traumatic wounds, and, in the absence of infection, if the body as a whole survives, these wounds will heal within a fairly definite period of time by the production of scar tissue. Whenever any accidental wound is made, an incalculable number and variety of organisms are introduced into the wound and they combine with the other foreign bodies and the injured tissue to interfere with wound healing. If they multiply and gain a foothold in the tissues and produce their poisons, they may damage more tissue and thus further interfere with the local and general effort on the part of the body to

repair the damage. When injured tissues are put at rest, unless there is some mechanical factor interfering with contact of the wound surfaces, these wounds will heal with a fairly uniform regularity and speed. The progress of wound healing and the strength of the repair follow a fairly constant curve for all tissues, as shown by Harvey[1] and Howes.[2] The restoration of normal tissue stability is complete at the end of about two weeks, except for such tissues as tendons or bone, which pull or hold heavy weights or loads. For these tissues also, there are fairly constant periods for repair, which have been well established. These periods are, of course, prolonged by any interference of the blood supply to the part or by certain chronic debilitating diseases, such as cancer. The length of time for the repair may be shortened on the other hand, by certain dietary regimens; for example, high protein feeding,[3] or high vitamin-C levels.[4,5] When the requirements for tissue growth and tissue repair are better understood, it may be possible to shorten greatly this interval of time.

The bête noire of wound healing in traumatic cases is infection. Micro-organisms call forth an exudate of fluid and cells that not only separate wound surfaces but also interfere with the metabolism of the regenerating cells.

Man in his conflict with animals and with his fellowman through the ages has also been subject to grave wounding These wounds have usually been more severe than accidental wounds because they were usually made with intent to kill and infection of these wounds has played a large part in the fatality. In the Middle Ages wounded men were usually quickly dispatched on the field of battle In more modern times many of the wounded died in hospitals or prison camps. As the nature and cause of wound infection became known, the mortality gradually declined, but even in the First World War there was an appalling loss of life from wound infection.

The approach of World War II was accompanied by a hope that wounds might be kept free from infection by the new chemotherapeutic agents that were yielding remarkable results in the treatment of infection. The most successful response to these sulfonamide drugs was in the field of medical infections characterized by a diffuse cellulitis without any breakdown of tissue. Drug administered systemically can reach such tissues by the blood stream and when resolution takes place the part is restored to normal. It was early observed that these drugs did not do so well in surgical infections characterized by a breakdown of tissue or a localized confined exudation of pus. Nevertheless it was hoped that they would be of great aid in the prevention of infection and would cut down the fatalities resulting from war wounds.

In 1940, the Surgeons General of the Army and Navy called upon the

National Research Council for advice regarding all of the latest developments in medical science that might be of aid to them in the prevention and treatment of disease and infection. Among other things, they asked for advice regarding the use of the newer chemotherapeutic agents Various committees and subcommittees were appointed to respond to this call. The Subcommittee on Surgical Infections applied itself to the problem of the prevention of infection in war wounds and burns. It was not a simple problem, to be settled on theoretical grounds.

It was deemed essential to carry out a well-controlled study of drug treatment in severe accidental civilian wounds simulating war wounds. The plan called for ten units in as many different cities, each unit to be fully equipped with clinical and laboratory facilities to care for and study cases of civilian accidental wounds, compound fractures, and burns that would simulate war casualties. All of the wounds were to be treated basically by as complete a surgical débridement as possible. The débrided tissue was to be sent to the laboratory for a complete analysis of the bacterial flora. A limited number of different kinds of local and general treatment, with a proper series of controls, were to be employed to appraise, if possible, the newer chemotherapeutic agents in the prophylaxis and in the treatment of wound infection. Careful observations and records were to be made of the course of wound healing, and particularly of any evidence of infection. If infection developed, its nature and etiology were to be determined by further laboratory studies and the cause of the failure of the preventive measures was to be analyzed. A carefully prepared summary sheet was planned to contain all of the available data from the record of the case that might indicate what factors favor or minimize the development of infection. These data could then be transferred to punch cards for statistical analysis.[6] After eighteen months, data had been accumulated from almost 2200 cases, and these were carefully analyzed by the author, with the following results.[7, 8]

I. THE GROSS RESULTS OF THE NATIONAL RESEARCH COUNCIL STUDY IN 1945

In the whole study, 2,191 cases were analyzed, of which 926 were soft-part wounds, 674 compound fractures, and 591 burns. Table I shows the incidence of serious and trivial infections in these main groups.

Each group was analyzed further by dividing it into two or three subgroups from the point of view of the presence or absence of certain common factors that might favor or minimize the development of infection. For example, all of the patients operated on within three hours of the accident were compared with those operated on after three hours, to bring out the difference between early and late operation. Similarly

TABLE I

INCIDENCE OF INFECTION IN THE WHOLE SERIES OF 2,191 CASES UNDER STUDY DIVIDED INTO THE THREE MAIN CATEGORIES

	Cases	Per cent Infection
SOFT-PART WOUNDS		
Total	926	
Serious infections	59	6.4
Trivial infections	104	11.2
		17.6
COMPOUND FRACTURES		
Total	674	
Serious infections	95	14.1
Trivial infections	77	11.4
		25.5
BURNS		
Total	591	
Serious infections	129	21.8
Trivial infections	146	24.7
		46.5

all of those with greater gross contamination of the wound as found at the time of operation were compared with those having lesser gross contamination. Likewise all of those with greater tissue damage were compared with those having lesser tissue damage—and so on with many relevant factors.

By gross contamination is meant the amount of dirt or other extraneous material the surgeon found in the wound at the time of the operation. By tissue damage is meant the amount of dead or injured tissue the surgeon found—classified as none, slight, moderate, and severe. The first two were considered lesser and the last two greater. These comparisons tend to bring out the importance of certain factors that we have long suspected as playing a role in favoring or minimizing wound infection The study, however, was undertaken *not* to weigh or measure the relative importance of these factors but *to find out what methods of treatment could prevent the development of infection.* In analyzing these cases, therefore, attention was focused primarily on the results obtained by the sulfonamide drugs.

The term 'infection' was defined for purpose of this study as 'the reaction of the tissues in and about the wound to the presence of micro-organisms or their poisons.' Infections were graded as serious or trivial—the former being those that showed general symptoms, or further destroyed tissue, or materially prolonged the period of wound healing or

hospitalization. The presence of infection and its severity were determined by all of the doctors in charge of the patient, and although there may have been a difference of opinion in borderline cases, the decisions must be considered reasonably accurate and consistent with the other evidence in the case. One may very properly say that it is only the serious infections that deserve consideration, but it must be remembered that the line between serious and trivial is not sharp. It is of interest to note, however, that the statistically significant differences are for the most part in the serious groups.

In this study, the criterion of infection was not the demonstration of bacteria in the wound, either at the start or later in the course of wound healing, but clinical evidence of the response of the body to the presence of those organisms.

A. SOFT-PART WOUNDS

The 926 soft-part wounds had an incidence of 6.4 per cent serious and 11.2 per cent trivial infections, or a total of 17.6 per cent. These figures indicate six or eight times as many infections as occur among a similar number of clean operative wounds in any well-ordered hospital. In the accompanying tables the cases have been divided into subgroups to bring out the importance of various factors.

Table II takes the group as a whole and divides it into either two or three subgroups to bring out some of the chief factors associated with infection.

It is seen that there was a high incidence of infection in multiple wounds, as compared with single wounds; in wounds that had greater gross contamination, as compared with those with lesser gross contamination; in those with greater tissue damage, as against those with lesser tissue damage; in those operated on more than three hours after they occurred, as contrasted with those operated on within that period; in large, as compared with small wounds; in those with incomplete, as against those with complete débridement, in long as compared with short irrigation, and in partial closure of the wound, as compared with complete closure or no closure. In most of these categories, we have confirmed statistically what we have learned from clinical experience regarding the main factors concerned with the development of, or resistance to, infection. It is of interest to note that significant differences in the total percentages of infection were found in all but one of the categories listed here. These differences were found either in the serious infections or in the trivial and sometimes in both. All of these groups needed further breakdown, for other factors play important roles in each of these categories that may weight the figures one way or another so as to disturb the comparability of the main groups. For example, one might expect the group with prolonged irrigation of the wound to include a larger number of

TABLE II

MAJOR FACTORS CONCERNED WITH WOUND INFECTIONS

	TOTALS	PER CENT INFECTION			
		None	Serious	Trivial	Total
Multiple wounds	300	75.8	7.7	S 16.5	S 24.2
Single wounds	626	85.4	5.8	S 8.8	S.14.6
Stab wounds	257 †	84.1	5.8	10.1	15.9
Greater gross contamination	322	76.0	S 10.3	13.7	S 24.0
Lesser gross contamination	604	85.8	S 4.3	9.9	S.14.2
Greater tissue damage	425	77.0	S 8.3	S 14.7	S 23.0
Lesser tissue damage	501	87.0	S 4.8	S 8.2	S 13.0
Operation after 3 hours	365 *	78.9	S 9.9	11.2	S 21.1
Operation before 3 hours	556 *	84.8	S 3.9	11.3	S 15.2
Large area	148	71.6	7.4	S 21.0	S 28.4
Small area	778	84.4	6.2	S 9.4	S 15.6
Incomplete débridement	270 *	76.6	S 9.3	S 14.1	S 23.4
Complete débridement	607 *	89.6	S 4.1	S 6.3	S 10.4
Incomplete removal of foreign body	70 *	81.4	7.2	11.4	18.6
Complete removal of foreign body	111 *	82.0	3.6	14.4	18.0
Irrigation for over 10 minutes	203	72.4	8.9	S 18.7	S 27.6
Irrigation for less than 10 minutes	723	85.2	5.7	S 9.1	S 14.8
No closure of wound	76	81.3	S 6.7	S 10.7	S 17.4
Complete closure of wound	759	85.9	S 5.5	S 9.9	S 15.4
Partial closure of wound	72	59.7	15.3	25.9	40.3

S. = Statistically significant differences
* In certain cases these data were not recorded
† Some stab wounds were single and some multiple

cases with maximum gross contamination than the group with a short period of irrigation; so these groups had to be subdivided and cross-tabulated.

Table III takes the group as a whole (without regard to the factors favoring infection) and divides it into controls, and the three main categories of drug-treated cases, together with a small group with miscellaneous drug treatments.

This table shows that the results in the three main categories of drug treatment yielded no significant differences. The very small series of miscellaneous treatments showed a significantly smaller number of infections, but for various reasons these cases were not comparable with the others, and one cannot conclude that the miscellaneous drug treat-

TABLE III

INCIDENCE AND PERCENTAGE IN SOFT-PART WOUNDS OF SERIOUS AND TRIVIAL INFECTIONS IN THE CONTROLS AND IN CASES OF PATIENTS WITH THE VARIOUS FORMS OF DRUG TREATMENT

	CASES	NUMBER OF INFECTIONS			PER CENT OF INFECTION		
		Serious	Trivial	Total	Serious	Trivial	Total
Total number of cases	926	59	104	163	6.4	11.2	17.6
Neither general nor local sulfonamide	322	15	25	40	4.7	7.8	S 12.4
Total sulfonamide	604	44	79	123	7.3	13.1	S 20.4
General without local	153	15	12	27	9.8	7.8	17.6
General with local sulfanilamide and sulfadiazine	187	8	26	34	4.3	13.9	18.2
General with local sulfanilamide	225	20	39	59	8.9	17.4	26.2
Miscellaneous sulfonamide	39	1	2	3	2.6	5.1	7.7

S. = Statistically significant differences.

ments are any better than the three regularly employed. This similarity of the three main drug groups ran through all of the cross-tabulations, and it seemed to be of particular interest that the combined general and local use of drug was no better than the systemic administration alone. With no difference in results shown in the three methods, it seemed justifiable to pool the whole drug-treatment experience, and consider it against the control experience. This comparison seems to indicate that the control patients did significantly better than the treated patients with a total of 12.4 per cent of infections, as compared with 20.4 per cent among the drug-treated patients. But further breakdown indicated that, as a group, the controls were somewhat less severely injured than the drug-treated cases. It was therefore necessary to cross-tabulate within these groups and compare subgroups having the same common factors. Such comparisons are shown in Tables IV and V.

In Table IV, the cases are divided in such a way that the drug-treated cases and controls can be compared within subgroups featuring certain factors thought to be of major importance with respect to the development of wound infection. It is seen that in every category, with one close

TABLE IV

SOFT-PART WOUNDS: COMPARISON OF CASES IN DRUG-TREATED PATIENTS AND CONTROLS WITHIN SUBGROUPS COVERING FIVE OF THE MAJOR FACTORS CONCERNED WITH WOUND INFECTION

MAJOR FACTORS	CONTROLS				SULFONAMIDE CASES			
	Totals 322	Per cent Infection			Totals 601	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
Greater gross contamination	103	7.8	10.7	18.5	219	11.4	15.0	26.4
Lesser gross contamination	S 219	3.2	6.4	9.6	385	4.9	12.0	16.9
Greater tissue damage	127	7.1	12.6	19.7	298	8.7	15.8	24.5
Lesser tissue damage	S 195	3.1	4.6	7.7	306	5.9	10.5	16.4
Incomplete or no débridement	98	10.2	7.1	17.3	221	8.6	15.4	24.0
Complete débridement	S 224	2.2	8.0	10.2	383	6.5	11.8	18.3
Irrigation more than 10 minutes	57	7.0	15.8	22.8	146	9.6	19.9	29.5
Irrigation less than 10 minutes	S 265	4.1	6.0	10.1	458	6.6	10.9	17.5
Late operation more than 3 hrs	38 *	10.5	13.2	23.7	96	11.5	10.4	21.9
Early operation less than 3 hrs	S 282 *	3.5	7.1	10.6	504	6.6	13.7	20.3

* Data missing in 6 of these cases
S = Statistically significant differences

exception in the smallest group, the total percentage of infections was higher in the drug-treated cases than in the controls. In all but one instance in each, this held true for both serious and trivial infections.

In Table v, two major factors are combined in a single group, thus lessening the chance of heterogeneity. In this table also was found a significant consistency. In all but one line of figures (which represents the smallest group), the percentage of total infections for the drug-

treated cases was higher than that of the controls. In two others, the serious-infection rate for the controls was slightly higher than for the drug-treated cases, but this difference was offset by a lower rate in the trivial infections. In only two categories were the numbers large enough and the differences great enough to be statistically significant and results were in favor of the controls, but the general trend was also significant and was *not* in favor of the sulfonamides.

Many more cross-tabulations were made, but they only confirmed the results in the ones presented above. No matter how one looks at these results, one cannot find any evidence to support the idea that the use of the sulfonamides lowered the incidence of infection in the accidental soft-part wounds covered in this study.

The question has been asked whether the use of the sulfonamides will delay the onset of infection. It was of interest to note the day of the development of infection and correlate it with the method of drug therapy (see Table vi).

Table VI

Soft-part Wounds: Day of Onset of Serious and Trivial Infections Correlated with the Drug Treatment and with the Controls

	Cases	Not Counted	Counted	Per cent of infections starting on days indicated			
				5 or Less	6-10 Days	11-15 Days	Over 15 Days
Total infections	163						
Serious	59	5 *	54	42 6	31 5	18 5	7 4
Trivial	104	18 *	86	27 9	43 0	15 1	14 0
No sulfonamide:							
Total infections	40						
Serious	15	1 *	14	50 0	28 5	14 3	7 2
Trivial	25	2 *	23	26 1	56 5	13 0	4 4
Sulfonamides:							
Total infections	123						
Serious	44	4 *	40	40 0	32 5	20 0	7 5
Trivial	79	16 *	63	23 6	38 2	15 9	17 5

* Time of onset of infection not recorded

When division was made into five-day periods, it was seen that the largest proportion of the *serious* infections developed in the first five days and the largest proportion of the *trivial* infections in the next five days. This was true both of the controls and the patients treated with drugs.

The figures for the first five days for both serious and trivial infections among the drug-treated patients were slightly lower than among the controls, suggesting that there was a slight delay in the onset of infection; but the differences were not statistically significant because the series were small.

Of primary importance, of course, are the kind and degree of bacterial contamination. An attempt was made in one unit to make a quantitative determination of the number of organisms present, but this was not found feasible. It cannot be claimed that all of the qualitative analyses of the bacterial flora were complete or that the rarer species of organisms were always properly classified, but the positive findings of the four main groups of pathogens seem reasonably accurate.

TABLE VII

SOFT-PART WOUNDS: INCIDENCE OF HEMOLYTIC STREPTOCOCCI AND COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS IN THE DÉBRIDED TISSUE, THEIR PERSISTENCE IN LATER CULTURES AND THEIR LATER APPEARANCE IN CASES IN WHICH THEY WERE NOT ORIGINALLY FOUND WITH PARTICULAR REFERENCE TO DRUG TREATMENT

	HEMOLYTIC STREPTOCOCCUS			COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS *		
	Débrided Tissue	Persisting	New	Débrided Tissue	Persisting	New
Total	55	11	18	74	16	51
Serious infection	9	4	6	7	3	14
Trivial infection	13	5	6	14	10	27
Controls						
Total	14	0	4	17	1	9
Serious infection	0	0	3	2	1	2
Trivial infection	2	0	1	1	0	6
Sulfonamide cases:						
Total	41	11	14	57	15	42
Serious infection	9	4	3	5	2	12
Trivial infection	11	5	5	13	10	21

* This does not include other coagulase positive micrococci.

Tables VII and VIII show not only the occurrence of these bacteria in the débrided tissue, but also the number of times these four groups of microbes persisted and the number of cases in which the organisms were found later though they had not been found originally—suggesting either

TABLE VIII

SOFT-PART WOUNDS: INCIDENCE OF PATHOGENIC AEROBIC GRAM-NEGATIVE BACILLI AND CLOSTRIDIUM WELCHII IN THE DÉBRIDED TISSUE, THEIR PERSISTENCE IN LATER CULTURES, AND THEIR LATER APPEARANCE IN CASES IN WHICH THEY WERE NOT ORIGINALLY FOUND, WITH PARTICULAR REFERENCE TO DRUG TREATMENT

	PATHOGENIC AEROBIC GRAM-NEGATIVE BACILLI			CLOSTRIDIUM WELCHII		
	Débrided Tissue	*Per-sisting*	New	Débrided Tissue	*Per-sisting*	New
Total	147	26	33	138	12	9
Serious infection	21	11	8	13	4	3
Trivial infection	20	8	19	19	3	4
Controls:						
Total	44	7	7	32	1	2
Serious infection	4	3	2	1	0	1
Trivial infection	3	1	5	7	1	1
Sulfonamide cases:						
Total	103	19	26	106	11	7
Serious infection	17	8	6	12	4	2
Trivial infection	17	7	14	12	2	3

a secondary contamination or the persistence of organisms originally present in such small numbers that they were missed in the first cultures. Furthermore, the presence, persistence, and new appearance of these organisms were all correlated with drug treatment and the presence or absence of wound infection. It would appear from these tables that the organisms disappeared as readily from the wounds of the control cases as from those receiving primary drug, and we have no indication whatsoever that the drugs primarily administered either locally or generally or combined were able to eliminate the organisms from the wounds. This is just as true of the hemolytic streptococcus and the *Clostridium welchii*, which are ordinarily considered susceptible organisms, as it is of the coagulase positive staphylococci and other micrococci and the pathogenic Gram-negative aerobic bacilli, which are more resistant.

In considering the tables showing the incidence of the different organisms during the course of treatment, one is struck by three things. First, the small proportion of cases in which pathogenic organisms were found in the original cultures of the débrided tissue, which later developed clinical infection. This is particularly striking with the *Clo-*

stridium welchii, which was found in 138 cases, but in only infection develop, and in only 2 of these was there clinical g Second, the rather small proportion of cases in which the or were found originally persisted in later cultures, for exam with the hemolytic streptococcus, two-ninths with the coagu *Staphylococcus aureus*, one-sixth with the pathogenic Gr bacilli, and one-eleventh with the *Clostridium welchii*. T indicate not only the thoroughness of the removal of the org with the débrided tissue, but also the ability of the body to the organisms left behind. Third, although in some cases the persisted in later cultures or appeared for the first time late not always evidence of clinical infection. This clearly indica criterion of infection was not the presence of pathogenic o the cultures but the clinical manifestations of their presence.

When the bacteriology is cross-tabulated with the closure o (see Tables IX and X), it is evident that in the closed wounds,

TABLE IX

SOFT-PART WOUNDS: CROSS-TABULATIONS BETWEEN BACTERIOLOGY A CLOSURE OF WOUND WITH PARTICULAR REFERENCE TO DRUG TR

	WOUND COMPLETELY CLOSED				
	HEMOLYTIC STREPTOCOCCUS			COAGULASE STAPHYLC AUREI	
	Débrided Tissue	Persisting	New	Débrided Tissue	Per sistir
Total	46	7	12	58	12
Serious infection	7	2	5	5	3
Trivial infection	12	4	5	9	8
Controls:					
Total	13	0	4	15	1
Serious infection	0	0	3	2	1
Trivial infection	2	0	1	1	0
Sulfonamide cases:					
Total	33	7	8	43	11
Serious infection	7	2	2	3	2
Trivial infection	10	4	4	8	8

organisms were frequently found in later cultures even though they were not recovered from the débrided tissue.

TABLE X

SOFT-PART WOUNDS: CROSS-TABULATIONS BETWEEN BACTERIOLOGY AND COMPLETE CLOSURE OF WOUND WITH PARTICULAR REFERENCE TO DRUG TREATMENT

	WOUND COMPLETELY CLOSED					
	PATHOGENIC AEROBIC GRAM-NEGATIVE BACILLI			CLOSTRIDIUM WELCHII		
	Débrided Tissue	Persisting	New	Débrided Tissue	Persisting	New
Total	121	16	22	106	6	7
Serious infection	13	6	6	7	2	2
Trivial infection	15	5	13	17	2	4
Controls:						
Total	40	6	7	29	1	2
Serious infection	3	2	2	0	0	1
Trivial infection	3	1	5	7	1	1
Sulfonamide cases.						
Total	74	10	15	69	5	5
Serious infection	0	4	4	7	2	1
Trivial infection	12	4	8	10	1	3

Note: Minor drug groups omitted

This suggests that they were there originally in too small numbers to be cultured and yet were able to gain a foothold in the tissues and grow out later. It is not likely, for example, that in many of the 12 closed cases in which the hemolytic streptococci appeared as new cultures they entered the wound as secondary contaminants, coming from the respiratory passages of attending doctors and nurses, however prevalent that may be with regard to open wounds as suggested by Colebrook,[9] Miles,[10] Hare,[11] and others. Furthermore, both the persistence of pathogenic organisms in wounds that were closed and their appearance as new cultures not found originally occurred just about as frequently in the cases primarily treated with drugs as in the controls

In spite of these far from satisfactory results in the prevention of infection in these accidental wounds by the use of the sulfonamides, there were only 4 deaths from infection in all of the 926 cases. What does

TABLE XI

SOFT-PART WOUNDS: SUMMARY OF 4 CASES, ALL WOUNDS CLOSED, IN WHICH DEATH FOLLOWED INFECTION. TOTAL CASES, 926

CASE	LESION	OPERATION	PRIMARY DRUG	SECONDARY DRUG	CAUSE OF DEATH	DAY OF DEATH	PRINCIPAL ORGANISMS
Case 1	Avulsion of scalp	Débridement, closure with tension	None	Sulfadiazine	Meningitis	24th	Coagulose-positive staphylococcus *Pseudomonas pyocyanea*
Case 2	Stab wound of chest, abdomen, diaphragm, and stomach	Débridement, closure without tension	Sulfanilamide locally, sulfadiazine generally	Sulfadiazine	Empyema	3rd	Hemolytic streptococcus Group F
Case 3	Gunshot wound of abdomen, perforation of ileum and cecum	Débridement, closure of perforations, closure of wound	Sulfanilamide in wound; sulfadiazine generally	Sulfadiazine	Peritonitis	3rd	*E. coli*, *C welchii*, nonhemolytic streptococcus
Case 4	Gunshot wound of abdomen, perforation of jejunum, rectum, and bladder	Incomplete débridement, closure of perforations, partial closure with drainage	Sulfanilamide in wound Sulfadiazine generally	Sulfadiazine	Peritonitis and pneumonia	5th	*E. coli*, green streptococcus

this signify? Perhaps the most plausible explanation is that the sulfonamides, although they have not been able to prevent or minimize local infections, were able to prevent the general spread of infection to the rest of the body. Of these 4 fatal cases, 3 were among the drug-treated patients and one was among the controls, but the drug-treated patients all had serious injuries that may have contributed to the fatal outcome. When infection developed among the controls, the worst cases were treated with sulfonamide as was done with the fatal case, so that we cannot say with certainty whether or not we should have had a higher incidence of sepsis and death among the controls if they had been carried through completely without drug (see Table xi).

For this reason, one has to fall back upon all past experience in order to say that this mortality from infection of 0.43 per cent is extremely low for these serious accidental wounds of the soft parts.

The results of this study should not be interpreted to mean that the sulfonamides have not an important place in the treatment of infection. However, that role is not in the prevention of local infection in the wound but in the prevention of local infection from getting out of control by becoming general and thus causing sepsis and death. The problem of the development of local infection in these wounds still remains unsolved and it will not be solved until we can find some way for the sulfonamides or some other agent to be effective in wounds in the presence of damaged tissue.

B. Compound Fractures

The problem of compound fractures has two aspects: one, infection; the other, bone healing and subsequent bone and joint function. However, this study by the Subcommittee on Surgical Infections of the National Research Council considered the problem only from the point of view of infection. The end result desired in compound fractures is a normally functioning member, with firm bony union and freely contracting muscle around the bone, and freely moving joints on either end of the fractured bone. This end result is materially altered or delayed by infection within or around the bone or by infection in the soft parts. Bone is less resistant to infection than are soft parts. It is frequently cut off from its blood supply either as a loose fragment or as the exposed end of the shaft that has been stripped of its periosteum. Infection may develop at once, within the first few days after the injury, or start up with the first efforts to obtain full motion of the part, or lie dormant for months after the wound has apparently healed and then suddenly or gradually develop after some trauma or some general lowering of resistance such as accompanies chilling or fatigue.

There were 674 compound fractures in the series studied, with 14.1

per cent serious and 11.4 per cent trivial infections, or a total of 25.5 per cent. Whereas there were twice as many trivial as serious soft-part wound infections, in compound fractures the serious exceeded the trivial. This was to be expected, because when bone becomes infected, wound healing is usually delayed and hospitalization is usually prolonged. In many of the trivial cases, only the soft parts were involved in the infection.

The compound fractures were divided into major and minor. The former included the large long bones of the upper and lower extremities, in which the problem of bone overriding and angulation from muscle contraction and the necessity for either internal or external fixation are of major importance. All of the others have been classified as minor.

Table XII shows the incidence of infection for each of the major bones. It is seen that the total number of cases as well as the percentage of infection is much higher in the lower extremity than in the upper, both bones of the leg being the most common lesion and giving the highest percentage of both serious and trivial infections, with the femur a close second.

TABLE XII

PERCENTAGE OF INFECTION IN COMPOUND FRACTURES OF THE DIFFERENT MAJOR LONG BONES

	TOTAL CASES	PER CENT INFECTION		
		Serious	Trivial	Total
Humerus	45	4 4	8 9	13 3
Radius	17	5 9	11 8	16.7
Ulna	28	7 1	3 6	10 7
Radius and ulna	40	10 0	7 5	17.5
Femur	30	23 3	10 0	33.3
Tibia	72	16 7	8 3	25 0
Fibula	11	9 1	0 0	9 1
Tibia and fibula	171	24 0	12 8	36 8
Totals	414	16 9	9.9	26 8

The principal factors concerned with the incidence of infection in compound fractures are shown in Table XIII. Here it is seen that shock, greater gross contamination, greater tissue damage, and large-sized wounds gave a significantly high percentage of serious infection. Other

TABLE XIII

MAJOR FACTORS CONCERNED IN THE INCIDENCE OF INFECTION IN COMPOUND FRACTURES

	TOTALS	PER CENT INFECTION		
		Serious	Trivial	Total
Shock	176 *	S 22.7	11.9	31.6
No shock	490 *	S 11.9	11.4	23.3
Greater gross contamination	316	S 21.4	10.1	31.5
Lesser gross contamination	328	S 6.4	12.8	19.2
Greater tissue damage	517	S 16.8	12.4	29.2
Lesser tissue damage	157	S 5.1	8.3	13.9
Wound area > 10 sq. in.	69 *	S 27.6	14.5	42.1
Wound area < 10 sq. in.	173 *	S 10.8	10.6	21.4
Wound completely closed	412	S 9.5	10.9	20.4
Wound partially closed	130	S 21.5	10.0	31.5
Wound left open	132	S 21.2	12.9	34.1
Plates	89	25.8	10.1	35.9
Plaster	346	16.8	10.1	26.9
Skeletal traction	116	21.8	14.8	36.6
Major fractures (large long bones of the extremities)	414	S 16.9	9.9	26.8
Minor fractures (all others)	260	S 9.6	13.8	22.4
Irrigation > 10 minutes	465 *	14.4	10.3	24.7
Irrigation < 10 minutes	207 *	13.0	14.0	27.0
Operation after 3 hours	304 *	15.8	12.5	28.3
Operation before 3 hours	362 *	11.9	10.8	22.7
Incomplete débridement	208 *	12.5	10.6	23.1
Complete débridement	451 *	13.3	11.1	24.4

S = Statistically significant differences

* Data missing on this point in a few of the cases.

factors are apparently less important, but all groups require cross-tabulation for an accurate appraisal. A surprising result is seen in the category of wound closure. The lowest percentage of serious infections is in the wounds that were completely closed, whereas for those partly closed or left open the percentage was high. This finding has been consistent throughout the course of the study, and it seems to be statistically significant. But it is generally recognized that wounds that were closed completely were as a group less seriously injured and were treated earlier than those left open. Another possible explanation for this is that when a compound fracture is left open or partly closed, the exposed bone, which is slow in being covered over with granulations, especially

when there are separated fragments, is subject to prolonged exposure to the secondary contamination of organisms that are able to gain a foothold.

Table xiv shows the compound fractures taken as a whole and divided according to the various forms of drug treatment. We find that the results with the three major methods of drug treatment do not differ significantly from one another nor from the controls, the serious infections among the drug-treated cases being a little higher and the trivial a little lower than the controls.

TABLE XIV

INCIDENCE AND PERCENTAGE OF SERIOUS AND TRIVIAL INFECTIONS IN COMPOUND FRACTURES IN THE CONTROLS AND IN THE CASES WITH THE VARIOUS FORMS OF DRUG TREATMENT

	CASES	NUMBER OF INFECTIONS			PER CENT INFECTION		
		Serious	Trivial	Total	Serious	Trivial	Total
Total number of cases	674	95	77	172	14.1	11 4	25 5
Neither general nor local sulfonamide	187	23	24	47	12.3	12 8	25.1
Total sulfonamide	487	72	53	125	14 8	10 9	25 7
General without local	104	13	7	20	12 5	6 7	19 2
General with local sulfanilamide and sulfadiazine	143	19	24	43	13 3	16 8	30 1
General with local sulfanilamide	209	36	13	49	17.2	6.2	23 4
Miscellaneous sulfonamide	31	4	9	13	12 9	29 0	41 9

When the percentage of cases in the control and drug-treated groups with the major factors favoring wound infection are compared, it is apparent that the two series are approximately equal in severity.

When comparisons are made between the controls and the drug-treated cases within the framework of the different factors that play a role in wound infection (Table xv), we find only one statistically significant difference, and that favors the controls. What is more significant is the fact that in those groups in which the factors favoring wound infection are maximal, the figures for the drug-treated cases in all but one instance exceed the figures for the controls. In those groups in

From Tables xv and xvi, it seems clear that in those situations in which local wound infection is favored to develop, the sulfonamides failed to demonstrate any definite prophylactic value.

TABLE XVI

COMPOUND FRACTURES: COMPARING THE DRUG-TREATED CASES WITH THE CONTROLS WITHIN SUBGROUPS COMBINING TWO OF THE MAJOR FACTORS CONCERNED WITH WOUND INFECTION

	187 CONTROLS				487 SULFONAMIDE CASES			
	Total Cases	Per cent Infection			Total Cases	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
Greater contamination and greater tissue damage	86	16.3	9 3	25 6	224	24 0	11.2	35.2
Greater contamination and lesser tissue damage	8	37 5	12 5	50 0	28	10 7	3 6	14 3
Lesser contamination and greater tissue damage	60	8 3	20 0	28.3	147	9 5	12 9	22 4
Lesser contamination and lesser tissue damage	33	3.0	9 1	12 1	88	1 1	9 1	10 2
Shock and greater tissue damage *	37	13 5	13 5	27 0	121	27 2	12 4	39 6
Shock and lesser tissue damage *	5	20 0	0 0	20 0	13	0 0	23 1	23 1
No shock and greater tissue damage *	107	13.1	15 9	29 0	247	13 8	11 7	25 5
No shock and lesser tissue damage *	36	5 6	11 1	16.7	100	4 0	6 0	10 0

* No data regarding shock on 8 summary sheets.

It is of interest to note the difference in the time of development of the infection in compound fractures as compared with that in soft-part wounds and to correlate this time factor with the use of drugs. This is shown in Table xvii. It is seen that the onset of infection frequently

Table XVII

Compound Fractures: Day of Onset of Serious and Trivial Infections Correlated with the Cases Receiving Drug Treatment and with the Controls

	Number of Cases	Not Counted	Counted	Per cent of Infections Starting on Days Indicated			
				5 or Less	6-10 Days	11-15 Days	Over 15 Days
Total infections	172						
Serious	95	9 *	86	21.0	21.0	23.0	35.0
Trivial	77	15 *	62	18.0	39.0	15.0	28.0
Controls:							
Total infection	47						
Serious	23	1 *	22	9.0	27.0	27.0	37.0
Trivial	24	0 *	24	25.0	50.0	17.0	8.0
Sulfonamide cases:							
Total infection	125						
Serious	72	8 *	64	25.0	19.0	22.0	34.0
Trivial	53	15 *	38	13.0	32.0	13.0	42.0

* Data regarding time of onset of infection are missing in these cases

was late, a large percentage of both serious and trivial infections appearing more than fifteen days after the fracture. The question has been asked whether the sulfonamides delay the development of infection in these wounds or render them less serious, but the table gives no evidence to that effect. In the patients receiving drug treatment, there was no lowering of serious infections as compared with the controls and, if anything, an earlier onset.

Of major importance in the development of infection in compound fractures is of course the presence of pathogenic bacteria. Cultures of all of the débrided tissue revealed many species, and it will require a special study to determine the significance of all of the organisms found. The tables shown herewith are concerned only with the four major pathogenic groups of organisms.

Tables XVIII and XIX show the number of cases in which the organisms originally found in the débrided tissue persisted in later cultures and the number of cases in which they appeared later as new cultures. All of these data are correlated with the method of treatment in the three major categories of sulfonamide therapy and in the controls. It is seen that the fractures were generally more highly contaminated with patho-

TABLE XVIII

COMPOUND FRACTURES: INCIDENCE OF HEMOLYTIC STREPTOCOCCI AND COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS IN THE DÉBRIDED TISSUE, THEIR PERSISTENCE IN LATER CULTURES, AND THEIR LATER APPEARANCE IN CASES IN WHICH THEY WERE NOT ORIGINALLY FOUND, WITH PARTICULAR REFERENCE TO DRUG TREATMENT

	HEMOLYTIC STREPTOCOCCUS			COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS *		
	Débrided Tissue	Persisting	New	Débrided Tissue	Persisting	New
Total	50	13	32	54	19	55
Serious infection	12	8	16	8	7	31
Trivial infection	13	2	8	9	7	11
Controls.						
Total	17	3	11	12	4	7
Serious infection	3	2	7	3	3	4
Trivial infection	5	0	3	1	1	0
Sulfonamide cases †:						
Total	31	9	20	40	15	48
Serious infection	8	5	9	5	4	27
Trivial infection	7	2	4	8	6	11

* Does not include other coagulase positive micrococci.
† Minor drug groups omitted

genic organisms than were the soft-part wounds, and a higher proportion persisted than in the wounds of the soft parts. A little over one-fourth of the hemolytic streptococci, a little over one-third of the coagulase positive Staphylococcus aureus strains, one-sixth of the pathogenic aerobic Gram-negative bacilli, and one-seventh of the C. welchii persisted. Thus it is evident that the débridement of the wound and the defense of the body rid the wound of the great majority of the contaminating organisms, but this held true no more for the sulfonamide-treated patients than for the controls. Furthermore, the use of the sulfonamides did not prevent pathogenic organisms from appearing anew *when they were not found in the débrided tissue, arising either from organisms present in such small numbers that they were not originally found or from organisms gaining a foothold as secondary* contaminants. This was true of the wounds that were closed as well as of those that were left open.

Table XIX

Compound Fractures: Incidence of Pathogenic Aerobic Gram-negative Bacilli and Clostridium welchii in the Débrided Tissue, Their Persistence in Later Cultures, and Their Later Appearance in Cases in Which They Were Not Originally Found with Particular Reference to Drug Treatment

	Pathogenic aerobic gram-negative bacilli			Clostridium welchii		
	Débrided Tissue	Persisting	New	Débrided Tissue	Persisting	New
Total	111	20	55	147	21	14
Serious infection	28	13	17	38	12	5
Trivial infection	11	1	31	17	2	4
Controls:						
Total	26	6	21	47	6	5
Serious infection	3	0	5	15	5	1
Trivial infection	4	3	11	3	0	3
Sulfonamide cases: *						
Total	78	11	28	92	13	8
Serious infection	23	11	10	20	7	4
Trivial infection	6	0	6	12	0	1

* Minor drug groups omitted.

In spite of the unsatisfactory record of the sulfonamides as preventives against local infection in these compound fractures, only two deaths from infection occurred (0.3 per cent) in the whole series. Each victim had suffered a severe injury, which played a part in the fatal outcome. Brief summaries of these cases are shown in Table xx. It is seen that each of the patients had local and general sulfonamide treatment and, although they died, they did not have septicemia. When infection developed in the control cases, sulfadiazine was generally administered, and in these cases there were no deaths and no septicemia. It seems fair to conclude, therefore, that although the sulfonamides failed to prevent local infection in compound fractures, they minimized the general spread of the local infection and may have reduced the number of deaths. But the problem of the prevention of local infection remains unsolved and will have to await the discovery of some new form of sulfonamide or some entirely new agent that will be bacteriostatic or bactericidal in the presence of damaged tissue.

Table XX

Compound Fractures Deaths from Infection—2 Cases in a Total of 67

Case	Bones	Operation	Primary Drug	Day of Death	Principal Organisms
Case 1	Tibia, fibula, ilium	Partial closure without tension. Plaster	Local sulfanilamide. General sulfadiazine	9th	Coag.-pos *Staphylococcus aureus*, green streptococcus, *Clostridium bifermentans*
Case 2	Femur, tibia, and fibula	Partial closure with tension Plate	Local sulfanilamide General sulfadiazine	110th	Coag.-pos *Staphylococcus aureus*, *Escherichia coli*, *Bacillus proteus*, *Pseudomonas pyocyanea*, *Clostridium sporogenes*

C. Burns

Severe burns may have five phases shock, toxemia and nitrogen imbalance, infection, slough separation, and repair. Although these five phases reach peaks of importance at different periods during the course of illness, they overlap to some extent Infection has always been a serious problem in burns. Since the recent development of better understanding and improved treatment for burn shock, infection has become of greater importance because of the large number of seriously burned individuals that have survived the shock phase Infection is of importance potentially from the moment of injury until the whole area is again covered with skin. Burns differ from other wounds in two important respects: first, they are usually extensive but not deep, whereas other wounds are relatively deep but not extensive, second, it is not often possible or advisable to remove the dead tissue at the first surgical procedure as it is with other wounds. These two facts are important from the point of view of infection, because the contamination with organisms is greater than in other wounds and impossible to eliminate, and the medium (dead tissue) that sustains their growth remains to favor their development The bacteria causing infection in burns may be those residing normally in the hair follicles or sweat glands or those deposited on the surface subsequent to the burn. A superficial burn caused by low temperature applied for only a short duration may not kill all the organisms in the hair follicles. A deep burn caused by a higher temperature applied for a longer time probably kills all of the bacteria in the skin in the central areas, but at the margin there must always be areas where the burn becomes superficial, and the organisms there remain capable of life and growth. In every case of burn, there are three zones· one in which the tissue is killed directly by the heat, one in which it is injured but not immediately killed, and the third in which it is not injured at all. The thickness of these zones depends on the degree of heat and the duration of its application and the location on the body. Some of the injured tissue later dies and some recovers. A separation takes place between the living and the dead, and the time of its separation depends on a number of factors of which infection is one.

In this study, the clinical observers tried to maintain a uniform conception of the meaning of the terms 'second' or 'third-degree' burns, and 'serious' and 'trivial' infections. A second-degree burn meant one in which the superficial epithelium was injured to the extent of blistering, and anything more severe than that short of a complete destruction of the deepest epithelial elements, so that restoration could take place from *residual* epithelium. A third-degree burn meant one in which all

of the epithelial elements over a given area were destroyed so that epithelial repair had to take place from the margin or from skin grafts. It is obvious that the degree of any burn cannot be determined when the patient arrives at the hospital, or indeed until the repair has taken place or has failed to take place, spontaneously. Furthermore, there cannot be a sharp line between a deep second-degree burn, where repair takes place from scattered residual islands of epithelium, and a superficial third-degree burn. In this study, burns that had any amount of third-degree areas were placed in this category, and an attempt was made to estimate as nearly as possible the per cent of body surface so involved. Similarly, in those that had only second-degree areas, the percentage of body surface burned was approximately determined according to the scheme of Berkow.[12]

With extensive surfaces involved, it is obvious that cultures taken from the surface will invariably reveal many kinds of organisms, both at the time of admission to the hospital and all during the course of the illness, but, in this study, burns were called infected only when, in the judgment of all of those caring for the patient, there was clinical evidence of a reaction of the body to the presence of organisms or their products. A serious infection was distinguished from a trivial infection when there was clinical evidence of a general, as well as a local, reaction of the body to the infection, further destruction of tissue by the infection, or a materially prolonged hospitalization as a result of the infection. In some cases, it was most difficult to decide whether or not infection occurred or whether it should be called serious or trivial, because there was often some reaction of the body, both local and general, to the presence of dead tissue during the course of its separation from the body.

When this study was initiated, there was a great diversity of opinion with regard to the best forms of treatment. These could be grouped in three main categories: (1) tannic acid,[13] (2) vaseline gauze with compression dressing,[14, 15] and (3) some form of sulfonamide.[16] Even these did not satisfy all of the clinical observers, and some desired to try out other frankly experimental methods. Although it was recognized that if the number of categories was large, the number of cases in each category would be small, it was felt that during the first year at least, certain latitude should be given each unit to try out new methods, with the proviso that at least two methods be consistently compared with each other in order to have some controls. In most of the units, some sulfonamide method was compared with a nonsulfonamide method. Beside the variation in the local form of treatment, the opportunity was offered either to administer or withhold general drug therapy. It was recognized that the hemoconcentration and the fall in blood pressure commonly occurring in severe burns would increase the risk of toxic effects from

kidney blockage with the sulfonamides. Therefore, the general administration of the drugs was done cautiously, particularly when large areas were covered by drug-bearing local applications. All of the foregoing circumstances diversified the methods of treatment in these cases, and yet some conclusions may properly be drawn from an assemblage and analysis of the data. Five hundred and ninety-one cases were analyzed, with serious infection observed in 21.8 per cent and trivial in 24.7 per cent, or a total of 46.5 per cent infections.

TABLE XXI

MAJOR FACTORS IN THE INCIDENCE OF INFECTION FOLLOWING BURNS

	TOTALS	PER CENT INFECTION	
		Serious	Trivial
Second and third degree	296	S 35 5	S 32 4
Second degree only	295	S. 8 1	S 17.0
Greater gross contamination	266	S 27 8	27.1
Lesser gross contamination	325	S 16 9	22 8
Greater tissue damage	390	S 27 2	S.28.5
Lesser tissue damage	201	S 11.4	S 17 4
Operation after 3 hours	149 *	24 2	27 5
Operation before 3 hours	431 *	20.7	24.2
Area over 10 per cent body surface	289	S 32 5	26 6
Area less than 10 per cent body surface	302	S.11.5	23.2
Shock	85 *	S 36 5	25 9
No shock	493 *	S 19 3	23 9

* Data missing in a few cases.
S. = Statistically significant differences.

Table XXI shows that the main factor in the incidence of infection in burns is the depth or degree of the burn. The cases were almost equally divided between purely second degree and those having some element of third degree, but the former group had only 8.1 per cent serious and 17.0 per cent trivial infections, whereas the latter had 35 5 per cent serious and 32.4 per cent trivial infections. Other major factors, as with soft-part wounds and compound fractures, were gross contamination, tissue damage, surface area, and shock.

When both the second- and the third-degree groups are divided into those treated with some form of sulfonamide and those receiving no drug in any form, we find that there is approximately the same number

of drug-treated and nondrug-treated cases in each group. Table XXII yields no evidence, however, that the use of drugs lowered the incidence of wound infection. In this table, the sulfonamide-treated cases are divided into three groups: general alone, local alone, and combined —and each one was studied for the incidence of infection in second- and third-degree burns. With one exception, all of the drug-treated groups had a higher incidence of infection than the nondrug-treated groups. That exception was in the smallest group of third-degree burns, namely, that which received 'local without general sulfonamide.' Here the percentage of serious infection was remarkably low. The low percentage was partly but not completely offset by a high percentage of trivial infections. This group was found to be largely composed of burns of small area, in both the second- and the third-degree groups, as is shown in Table XXIII. This table also brings to light the fact that the controls had a lower percentage of extensive burns than the drug-treated cases in both the second-degree (26 per cent to 29 per cent) and in the third-degree (15 per cent to 21 per cent) groups, but these differences are not statistically significant.

When the drug-treated and nondrug-treated groups are compared further within the subgroups pertaining to the major factors concerned with infection, the results are of interest. They appear in Table XXIV. In each of the categories, the numbers were about equally divided except for shock. (Shock *per se* may be a factor favoring infection, but it has been thought that the *factors producing shock* are more important in that regard.) In spite of this apparent weighting of the sulfonamide group with more serious cases, the percentage of infection in the shocked cases was considerably lower than in the other groups, thus apparently favoring sulfonamide treatment, but the difference is short of statistical significance, for the series is small. There seems to be also a preponderance of cases with greater contamination in the sulfonamide group, but here the percentage of infection favors the controls, and this difference is on the borderline of significance. In what is perhaps the most important category, namely 'greater tissue damage,' the numbers were almost equally divided both for the number of cases and for the percentage of infections.

The figures for area are of considerable interest. Here the total areas are given, and in each group the figures for the drug-treated cases were higher than for the controls. These differences would not of themselves be significant, except that they all point in the same direction, which is in favor of the controls. When the factors of extent and depth in the third-degree burns (see Table XXV) were combined, it was found that in the smaller-area burns, the drug-treated and nondrug-treated cases were almost identical both in numbers of cases and the percentage of

TABLE XXII

COMPARISON OF SULFONAMIDE-TREATED CASES AND NONDRUG-TREATED CONTROLS IN SECOND- AND THIRD-DEGREE BURNS

	SECOND DEGREE				THIRD DEGREE			
	Number of cases	Per cent Infection			Number of cases	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
No sulfonamide	158	7.0	17.1	24.1	142	35.2	30.3	65.5
Sulfonamide	137	9.5	16.8	26.3	151	35.7	34.4	70.1
General without local sulfonamide	27	11.1	18.5	29.6	56	35.7	35.7	71.4
Local without general sulfonamide	37	5.4	21.6	27.0	34	8.8	47.1	55.9
Both general and local sulfonamide	73	11.0	13.7	24.7	61	50.0	26.6	76.6

TABLE XXIII

BURN CASES RECEIVING LOCAL AND NOT GENERAL SULFONAMIDE WERE GENERALLY LESS EXTENSIVE THAN THE OTHER SULFONAMIDE GROUPS, BUT THE DRUG-TREATED CASES AS A WHOLE WERE SOMEWHAT MORE EXTENSIVE THAN THE CONTROLS

	SECOND DEGREE		THIRD DEGREE	
	Less Than 10 Per cent Body Surface	10 Per cent or More Body Surface	Less Than 10 Per cent Body Surface	10 Per cent or More Body Surface
No sulfonamide	117	41	121	21
Sulfonamide	97	40	122	32
General without local sulfonamide	14	13	45	11
Local without general sulfonamide	32	5	30	4
Both general and local sulfonamide	51	22	47	17

TABLE XXIV

COMPARISON OF BURN DRUG-TREATED CASES AND CONTROLS WITHIN THE SUBGROUPS THAT INCLUDE THE MAJOR FACTORS CONCERNED WITH WOUND INFECTION

	300 CONTROLS				291 SULFONAMIDE CASES			
	Total Cases	Per cent Infection			Total Cases	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
Second degree only	158	7.0	17.1	24.1	137	9.5	16.8	25.3
Second and third degree	142	35.2	30.3	65.5	154	35.7	34.4	70.1
Greater contamination	116	25.8	22.4	48.2	150	29.4	30.6	60.0
Lesser contamination	184	16.7	23.9	40.6	141	17.0	21.2	38.2
Greater tissue damage	192	28.6	26.0	54.6	198	25.6	30.6	56.2
Lesser tissue damage	108	5.6	18.5	24.1	93	18.3	16.2	34.5
Operation after 3 hrs *	81	17.3	29.6	46.9	68	32.4	25.0	57.4
Operation before 3 hrs *	200	20.6	22.0	42.6	222	20.7	26.1	46.8
Shock	24	50.0	20.8	70.8	61	31.2	27.9	59.1
No shock	270	17.4	23.3	40.7	223	21.5	24.7	46.2
Total burned area of body surface								
Less than 5 per cent	75	2.7	12.0	14.7	66	6.1	15.2	21.3
5-9 per cent	74	*16.2*	33.8	*50.0*	69	21.5	31.9	53.4
10-19 per cent	93	22.6	24.7	47.3	87	26.4	29.9	56.3
20 per cent or over	58	38.0	24.2	62.2	69	45.0	24.6	69.6

* Data missing on 11 cases.

infections, whereas in the most extensive group, there were more patients who received the drug and yet they had a lower percentage of infections. However, when only those patients who lived long enough to have grafting done were counted, the difference was not so great and fell short of statistical significance. *In all of these comparisons,* therefore, there is no clear-cut evidence of the value of the sulfonamides in the prevention of infection in burns except possibly in the most seriously and extensively injured group.

TABLE XXV

COMPARISON OF SULFONAMIDE-TREATED BURN CASES AND NONDRUG-TREATED CONTROLS IN THE SUBGROUPS OF THIRD-DEGREE BURNS DIVIDED ACCORDING TO THE AREA INVOLVED WITHOUT REGARD TO THE EXTENT OF THE ASSOCIATED SECOND-DEGREE AREAS

		CONTROLS				SULFONAMIDE CASES		
	Total Cases	Per cent Infection			Total Cases	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
3rd degree burn area of body surface:								
Less than 5 per cent	89	18.0	34.8	52.8	89	31.5	36.0	67.5
5-9 per cent	32	56.3	25.0	81.3	33	42.4	36.4	78.8
10 per cent or over S	21	76.2	19.0	95.2	32	40.6	28.1	68.7
Grafted cases.								
Less than 5 per cent	20	35.0	55.0	90.0	35	45.7	40.0	95.7
5-9 per cent	26	65.4	23.1	88.5	21	52.4	42.8	95.2
10 per cent or over	13	77.0	23.0	100.0	21	47.7	33.3	81.0

S. = Statistically significant differences

When two of the factors were combined, as in Table XXVI, there were no differences of statistical significance except for one small group, which favored the controls. In other categories, there was little to choose between the two groups.

In this study, no one method of local treatment stood out above the others but, *taken as a whole, the compression-dressing method was* found to be superior to the eschar-forming treatments.

The day of onset of infection was of interest as is shown in Table XXVII. The time of onset was not noted in some of the early cases, but there were data of this kind for 232 of the 275 cases that developed infection The cases were divided into those receiving sulfonamide and those without sulfonamide, and each of these main groups was subdivided into five-day units. It is seen from the table that in the whole series, the peak of the serious cases developed in the second five-day period, whereas in the trivial cases, the onset was fairly evenly distributed through the first fifteen days. There is no indication from the

TABLE XXVI

BY CROSS TABULATIONS A COMPARISON OF DRUG-TREATED BURN CASES AND CONTROLS WITHIN THE SUBGROUPS COMBINING TWO OF THE MAJOR FACTORS CONCERNED WITH THE DEVELOPMENT OF INFECTION IN BURNS

	CONTROLS				SULFONAMIDE CASES			
	Total Cases	Per cent Infection			Total Cases	Per cent Infection		
		Serious	Trivial	Total		Serious	Trivial	Total
Greater contamination and greater tissue damage	95	30 5	26 4	56 9	124	26 6	34.7	61 3
Greater contamination and lesser tissue damage	21	4 8	4 8	9 6	26	42 3	11.5	53 8
Lesser contamination and greater tissue damage	97	26 8	25 8	52 6	74	24 3	24 3	48 6
Lesser contamination and lesser tissue damage	87	5 8	21 8	27 6	67	8 9	17.9	26.8
Greater contamination and shock	15	46 7	23 9	70.6	42	31.0	31 0	62 0
Greater contamination and no shock	99	22 2	22 2	44.4	103	29 1	29 1	58 2
Lesser contamination and shock	9	55 5	22 2	77 7	19	31.6	21.0	52 6
Lesser contamination and no shock	171	14 6	24 0	38 6	120	15 0	20 8	35 8

figures on the table that infection in the sulfonamide-treated patients was delayed by this treatment or that the infection was less serious than in the nondrug-treated cases.

The bacteriology of burns covers a wide range if a careful analysis is made of the bacterial flora present in the loose skin removed from the burned area. Swab cultures are entirely inadequate, and even the cultures of all of the tissue removed cannot reveal with certainty all of the organisms present in any given case. For this report, interest was centered around the well-known pathogenic organisms, namely, the hemolytic streptococcus, the coagulase positive staphylococci, the

TABLE XXVII

TIME OF ONSET OF THE INFECTION AS CORRELATED WITH THE USE OR NONUSE OF SULFONAMIDE TREATMENT IN 232 OF THE 275 CASES OF INFECTION FOLLOWING BURNS

	NUMBER OF CASES	NOT COUNTED	COUNTED	PER CENT OF INFECTIONS OCCURRING IN EACH 5-DAY PERIOD			
				5 Days or Less	6-10 Days	11-15 Days	More Than 15 Days
Total infections	275						
Serious	129	22 *	107	25.2	39.3	15.0	19.6
Trivial	146	21 *	125	28.8	28.8	31.2	11.2
Controls:							
Total infection	131						
Serious	61	5 *	56	23.2	46.4	8.9	21.5
Trivial	70	9 *	61	27.0	27.0	36.0	8.2
Sulfonamide cases:							
Total infection	144						
Serious	68	17 *	51	27.5	31.4	23.5	17.6
Trivial	76	12 *	64	29.7	29.7	26.6	14.0

* Data regarding time of onset of infection are missing in these cases

Gram-negative bacilli of the escherichia, aerobacter, typhoid-dysentery, pyocyaneus and proteus groups, the *Clostridium welchii*, the anaerobic streptococci, and other pathogenic anaerobes.

Particular interest lies not only in the incidence of these organisms in the débrided tissue but also in the persistence of these organisms in the cases during the course of treatment and their later appearance as apparently new cultures in cases in which they were not originally found. These figures, which appear in Table XXVIII, were broken down to show the influence of the sulfonamides on this persistence and new development.

One is struck by the large number of cases in which these organisms were originally found that failed to develop infection, and also by the comparatively large number of cases in which they appeared as new cultures. It is equally obvious from these charts that the primary use of sulfonamides was not associated with any more thorough elimination of these organisms than in the control cases, nor did they prevent the establishment of the organisms in the wound.

TABLE XXVIII

INCIDENCE OF CERTAIN OF THE PATHOGENIC BACTERIA IN THE DÉBRIDED TISSUE IN BURNS, THEIR PERSISTENCE IN THESE CASES AND THEIR APPEARANCE IN OTHER CASES IN WHICH THEY WERE NOT ORIGINALLY FOUND, WITH PARTICULAR REFERENCE TO SULFONAMIDE TREATMENT AND THE DEGREE OF THE BURN

	SECOND DEGREE			THIRD DEGREE		
	Dé-brided Tissue	Per-sisting	New	Dé-brided Tissue	Per-sisting	New
Hemolytic streptococcus						
No sulfonamide	15	3	6	16	5	25
Sulfonamide	21	2	3	25	7	22
Coagulase positive *Staphylococcus aureus*						
No sulfonamide	13	6	8	22	13	16
Sulfonamide	27	9	11	20	11	36
Pathogenic aerobic Gram-negative bacilli						
No sulfonamide	32	5	10	33	11	45
Sulfonamide	33	4	10	45	21	41
Clostridium welchii						
No sulfonamide	40	6	1	40	5	1
Sulfonamide	46	1	1	49	5	4

During the first year, it was particularly evident that the anaerobes were more easily eliminated from these burns than were the aerobes. Apparently the surface of dead skin does not offer the same favorable environment for growth and invasion as do the muscular layers. It was therefore decided at the end of the first year to omit the anaerobic cultures on burns unless infection developed. No anaerobic cultures were taken in 204 of these burns. The incidence of anaerobes in the débrided tissue is therefore based on 287 cases, anaerobes being found in 261.

In spite of the apparently questionable value of the sulfonamides in the prevention of local infection in burns, there were only 3 deaths from infection in the whole series—0.5 per cent. Brief abstracts of these cases are shown in Table XXIX. These were all second- and third-degree burns with 50 per cent or more of the body surface involved. Two were primarily treated with general sulfadiazine and one of these with local sulfonamide as well. Neither developed septicemia. The third had no general drug. Tannic acid had been used before admission, but after

Table XXIX

Deaths from Infections, 3 Cases in a Total of 591 Cases

Cases	Degree	Region	Per cent of body surface	Local treatment	Primary general drug	Secondary general drug	Day of death	Principal organisms
Case 1	Second and third	Face, trunk, both arms and hands, right leg	60-70	Equal parts powdered sulfanilamide and sulfadiazine	Sulfadiazine	Sulfadiazine	5th	Coag.-pos. staphylococcus, nonhemolytic strept., *Ps. pyocyanea*, *B. proteus*
Case 2	Second and third	Face, trunk, and all limbs	60-70	Fibrinogen, thrombin, sulfadiazine	Sulfadiazine	Sulfadiazine	28th	Coag.-pos. staphylococcus; hemolytic strept., group D, *Ps. pyocyanea*, *E. coli*
Case 3	Second and third	Face, trunk, upper extremities, and thighs	40-50	Tannic acid before admission. Vaseline with pressure after admission	None	Penicillin	24th	Coag.-pos. staphylococcus (septicemia); hemolytic strept., group G; *B. melaninogenicum*

admission, the local treatment was changed to vaseline gauze and compression. *Staphylococcus aureus* septicemia developed in the fifth day and failed to respond to penicillin, although death did not occur until the twenty-fourth day. This may be considered a low mortality from infection in burns. When the controls became seriously infected, sulfonamides were often used systemically, and we therefore have no control series carried through the whole course of treatment to compare with the mortality figures with the sulfonamide-treated patients.

The sulfonamides have apparently been useful in cases of infection by reducing the spread of the infection, and even in some cases that were not originally treated with the drug. There is no indication from this study that these drugs should be used as prophylactic agents in cases of burns.

D. THE PROPHYLAXIS OF INFECTION WITH SULFATHIAZOLE MICROCRYSTALS AND PENICILLIN

The study of the prevention of infection in accidental wounds and burns was continued for one more year in four of the units to appraise the microcrystals of sulfathiazole and penicillin. Two new units were set up to appraise penicillin in compound fractures, and six units continued the study of burns. It was thought that the failure of sulfanilamide and sulfadiazine to reduce materially the incidence of infection in the first eighteen months of the study might have been due to the fact that they were not applied until after débridement, whereas many of the war casualties were receiving the drugs immediately or promptly after the accident, often before the bacteria had had an opportunity to multiply. The microcrystals of sulfathiazole had been used and highly recommended by Ferguson[17, 18] after his experience on a hospital ship in the Pacific. His series had no controls, but his results were impressive and warranted checking. There was some doubt whether his patients were really representative of the front-line casualties or comparable to the day-by-day battle injuries.

The combined data from all four units studying soft-part wounds and compound fractures seemed to indicate that the microcrystals of sulfathiazole gave a lower incidence of infection than other forms of sulfonamide, but the results were not significantly better than the nondrug-treated controls.

Penicillin, used by one unit in soft-part wounds, yielded a definitely lower figure than the controls, but in this group the inhibitors of penicillin action—namely the Gram-negative bacilli—were strangely lacking, and it is questionable whether they were truly representative of battle casualties or the general run of civilian accidents in which these intesti-

nal organisms are so commonly found as 'fellow travelers' of the pathogenic cocci.

Penicillin, still scarce when the study was begun, was released by the Committee on Chemotherapy and other agents only for the prevention of infection in compound fractures of the large, long bones. The two new units together with the four already under way accumulated and observed 109 cases. These were controlled by cases treated with microcrystals of sulfathiazole and other sulfonamides and cases without drug, but the combined data gave no clear indication that either penicillin or the microcrystals of sulfathiazole had the power to prevent infection in compound fractures.

Further studies must be carried out with penicillin, but because of its weakness in mixed infections, which is attributable to the fact that so many organisms commonly present in such lesions as well as in accidental and war wounds are able to inactivate the antibiotic, too great reliance must not be placed upon it in this situation. Certainly it can be stated without fear of contradiction that in the treatment of civilian accidental wounds or battle casualties, no antibacterial agent now available can make up for the neglect of sound surgical principles. The prevention of infection depends upon prompt operation as soon as possible after the injury is received, the complete removal of dead tissue, loose bone fragments, and foreign bodies; the maintenance of good blood supply and adequate rest for the injured part. The use of the sulfonamides and penicillin has unquestionably minimized the generalized spread of infection from the site of injury, but has not significantly diminished the incidence of local infection. In burns, the figures seem to indicate that secondary contamination is of very great importance in the development of infection, and there is an urgent need for the discovery of some antibacterial agents that may be used locally to inhibit the growth of either primarily or secondarily contaminating pathogenic and nonpathogenic bacteria

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The Organization of an Adequate Bacteriological Service for a Surgical Department

I. THE NEED

THE FOREGOING chapters clearly indicate that what we call 'modern surgery' began when the science of bacteriology was born. As soon as it had been clearly demonstrated that bacteria were the cause of the disabling and often fatal infections that occurred in operative wounds, and that they could be prevented from entering the wound if certain precautions were taken to close the various doors by which they might get in, modern surgery was permitted to grow rapidly. Surgery must not lose sight of the fact that it depends for its very existence on the science of bacteriology. They began together and it is essential that they should stick together for their mutual protection and for their mutual advantages. It is true that the widening of the scope of 'modern surgery' required the surgeon to prolong and perfect his study of anatomy and develop his technical skill. Many surgeons have given so much time and attention to these features of their calling that they have forgotten that they must also keep abreast of the times in bacteriology or they will fall into careless habits and find infections developing in their clean operative wounds or infections that have become so established failing to come under their control.

If one makes rounds on a general surgical service, one finds that from a third to half of the cases are problems of infection either potential or established, that is, bacteria have either contaminated the body of the patient just before admission to the hospital through some accidental wound or burn; or bacteria have invaded certain organs or tissues of the body, having entered through some internal or external portal at some previous time. Furthermore, all cases that come to operation, as mentioned before, become contaminated in the operating room to a greater or lesser extent and may be candidates for infections.

In a large hospital or on a surgical service with a large staff, certain types of cases are often delegated to one man so that he may become expert in their care. Thus we may have those particularly trained in

cancer, in injuries, in the gynecological field, in fractures, in congenital anomalies, in plastic problems, and in infections. In a small hospital, the surgeon has to have knowledge in all of these fields. In a large hospital, the specialists in one field may be quite inexperienced in another field. Yet all surgeons must have some knowledge and interest in bacteriology because of the potential danger of infection in all fields of surgery.[1]

Consequently, every first-class surgical department and every accredited hospital should have available a bacteriologic service adequate to its needs. This service must be fourfold and ready for: (A) routine work, (B) emergency work, (C) special minor research, and (D) major research.

A. Routine Work

First of all, there is a great mass of routine service to be rendered—the periodic check-up of the operating-room technic, the autoclaves, the instrument sterilizers, the water sterilizers, the antiseptics, the catgut; the ward-dressing technic; the cultures of infected wounds, abscesses, carbuncles, empyemas, sore throats, urine, and other material obtained from the daily run of operative cases and ward dressings. This work is perhaps the easiest to handle. It can be done in the course of the day's work between nine and five and a large mass of material may be handled at the same time.

B. Emergency Work

Secondly, and perhaps more important, there is a great quantity of work that might be called emergency bacteriology, to be done on short notice any time of the day or night. A patient comes into the hospital with peritonitis. The surgeon operates and finds a large amount of free peritoneal fluid without any evident perforation of the gut. He should know as soon as possible the nature of the infection. A smear and an immediate examination of the stained slide must be made while he is still operating, and cultures must be made at once so that he may know within the time of brief incubation the organisms involved. Or a patient comes in with a compound fracture, and it is vitally important for the surgeon to know as soon as possible what organisms are contaminating the wound and are potentially dangerous. The débrided tissue must be analyzed, and the presence of pathogenic organisms reported within twenty-four to forty-eight hours. Not infrequently, after the fractured bone has been set up in plaster or with the proper traction and suspension apparatus, suddenly in the middle of the night the temperature goes up and the patient complains of pain in the wound. It is found to be swollen, edematous, a little dusky. There is doubtful crepitation of the tissues. The surgeon must know

promptly from smear and culture whether the gas bacillus is at work and which of the four pathogenic species is present. Suppose a patient has been operated upon for a pyloric obstruction, a gastroenterostomy has been done; cough and hiccough are annoying postoperative complications. Suddenly in the night he develops pain in the wound. An examination reveals puffiness and crepitation beneath the wound. The surgeon must know at once whether a gas infection has developed or gas from a perforation or leak has appeared in the wound. A quick bacteriological examination is necessary, a stained smear of the exudate often indicating the activity of organisms in such a wound. A patient comes in with a high fever and is obviously septic, the focus is not apparent, but it is important to know as soon as possible whether or not the patient has a positive blood culture. Let us say that a patient is admitted to the hospital with high fever and a swollen, painful, red, hot knee joint. The surgeon aspirates the fluid and needs to know by smear and culture the nature of the infecting organism. Any delay will materially affect the promptness of treatment and the eventual outcome. A patient is admitted with signs of fluid in the chest, aspiration reveals a thin, turbid fluid. Is it transudate or exudate? What bacteria are present? The correctness of treatment depends upon the prompt answer to these questions.

C. Special Bacteriological Minor Research

Many patients who come into the hospital present unusual problems in surgical infection. The case may be an unusually severe type of a well-recognized infection, or a rare type not easily recognized. Obviously the case needs some special bacteriological study. Routine methods of taking specimens or routine methods of cultivation will not suffice. The preliminary culture may find no organisms in a lesion, obviously an infection; it may reveal an unusual organism with peculiar properties requiring further study, or it may yield a mixture of organisms, only one of which is significant Such problems come up in severe cases of septicemia in which not only must the diagnosis be made, but also the course of the disease must be followed from day to day. Serious involvement of the face and neck following the spread of an infection from teeth, tonsils, or esophagus requires this special kind of bacteriological service, for here we may have a mixture of mouth organisms, and the severity of the infection may be due not so much to any single species of organism present as to the synergism of the various species. Such complex problems likewise arise in cases of acute or chronic infective gangrene of the skin, which not only may have to be handled by means of a special bacteriological analysis, but also may require a series of animal experiments before the nature of the infection can be deter-

mined and the problem satisfactorily solved. Cases of lung abscess or bronchiectasis require a complete bacteriological analysis before they can be intelligently handled. Liver abscesses frequently need to be studied from several different angles before the cause can be determined and the appropriate treatment applied. Chronic ulcers of the abdominal wall or of the extremities often present difficult bacteriological problems, which cannot be solved by simple procedures. Certain extensively infected wounds with loss of substance such as are seen frequently in time of war and occasionally in cases of civilian accident require not only an analysis of the original bacterial flora but also a periodic examination to determine when the various organisms disappear and when it is safe to subject the wound either to skin graft or secondary closure; for a failure to consider the bacteriological elements in such a problem may result in a failure of the surgical procedure. Unless such unusual problems are satisfactorily solved, the bacteriological service cannot be called adequate.

D. MAJOR RESEARCH

Besides the minor researches that may be required in connection with any individual case, there are certain major or perennial problems in bacteriology in which surgeons should be interested. Some of these they very properly share with the internist; for example, the problem of pneumonia. Although the pathogenesis of postoperative pneumonia may have a different mechanism from ordinary pneumonia, the bacteriological aspects may very well be the same in both cases. Lung abscess and empyema are of interest to both the medical and surgical services, but here it must be admitted that the surgeon is more directly concerned. In like manner, both surgeon and internist are involved in bacterial problems associated with the alimentary canal. The internist is most concerned when the disease is the result of a disorder caused by the organisms while they remain within the tract, whereas the surgeon becomes particularly interested when the intestinal organisms invade the tissues outside the alimentary tract, for example in peritonitis, cholecystitis, cholangiitis, pylephlebitis, and so on. Arthritis may be a problem in which the internist is more concerned, but when it becomes a suppurative process the surgeon must take a hand. The surgeon is taking more and more interest in the disease of the peripheral blood vessels, more perhaps from the point of view of the vasomotor nervous mechanism than from the bacteriological point of view, but we still have the problems of suppurative phlebitis, postoperative thrombophlebitis with pulmonary embolism, migrating phlebitis, and thromboangiitis obliterans, which have not yet been satisfactorily studied from a bacteriological point of view.

to infection. The factors which determine the formation of antibodies are not clear. Why is immunity transient to some types of infection and permanent with others? It has been suggested that antibody formation occurs only as long as direct antigenic stimulation occurs. This is an interesting theory, but it must be proved.

The problem of infection in the diabetic is of especial interest. The explanation for the peculiar susceptibility to infection in diabetes was ascribed by older writers to the increased sugar content of the blood and tissues. Studies along this line have not aided in solving the question and there is still no adequate solution. This problem has great practical importance not only because of the seriousness of infection in diabetics, but also because of the greatly increased risk of operating upon diabetic tissues.

The role of intestinal bacteria and bacterial products in the toxemia of intestinal obstruction is still uncertain. The amount of absorption from intestinal obstruction and distention has been a matter for debate and experimentation for a long time, but the cause of death in non-strangulated intestinal obstruction, apart from that in high obstruction, is still unknown. The facts are little known, but the possibility of a large bacterial factor in the production of the clinical symptoms cannot be overlooked.

Likewise, the importance of sepsis in the toxemia of burns is a much disputed question. Whether or not this toxemia is caused by the altered blood chemistry secondary to the pouring of large quantities of plasma into the burned tissues, or the absorption of a toxin or toxins from the burned area, or from bacterial sepsis is not clear.

Peritonitis continues to present many perplexing problems. In the past we have usually been concerned with the more obvious and more easily cultured bacteria recovered from peritonitis. With the development of better media and means of cultivation, many other types of organisms, particularly anaerobic, have been consistently found in peritoneal exudates. There is reason to believe that this disease represents a very complex process caused by the combined activity of many types of bacteria The toxemia of peritonitis has not been explained. It has been assumed that the absorption of bacterial products is responsible for the clinical symptoms, although the nature and action of these products are still unknown factors

The indications and limitations of the various sulfonamides in the treatment of infections will be dealt with in Chapter XVI, but the success of these drugs has given a stimulus to further research in the field of chemotherapy. The synergism of bacteria is a fascinating interest, but the antagonism of bacteria may be of even greater practical importance. Penicillin is just the beginning of success in this field. Many gaps are left that must be filled and the study of mixed infections becomes of increasing importance since that was found to be the weak spot in the defensive strategy of penicillin.

II. THE SOLUTION

A. THE LABORATORY

In going about the hospitals in New York City and its suburbs to see various types of surgical infections, one finds that often the most obviously necessary bacteriological procedures have been neglected. Anaerobic bacteriology has been particularly neglected. In 1937, a questionnaire was sent to all of the hospitals in the city asking them particularly whether they were equipped to perform anaerobic cultures and how often the laboratory was called upon by the surgeons to perform this function. Out of 137 hospitals in the city, 76 responded to the questionnaire. This included all of the larger hospitals. Presumably the other 61 had little or no facilities and little or no interest in the question. Of the 76 that replied only 8 claimed to be fully equipped for anaerobic bacteriology, 6 others were fairly well satisfied that they could handle this work, 37 indicated that they were not satisfactorily equipped, and of the other 25, 15 had no bacteriological laboratories at all. Thirteen of the hospital laboratory heads stated that the surgical department frequently called upon them for such culture. Eleven reported that there were occasional requests. In 34 they were made rarely, and in 18, never.[3] I am sure that this is not a condition peculiar to New York. Articles about surgical infections, even in our best surgical journals, are filled with evidences of inadequate bacteriology in connection with the cases reported, and frequently the surgical authors make apologies for the bacteriology done in their own hospitals. It is partly the surgeons' fault. If they want good surgical bacteriology enough they will demand and get it.

The attention of the Hospital Standardization Committee of the American College of Surgeons was called to this situation, and they were asked to look into the matter and consider the disapproval of those hospitals not fully equipped for adequate surgical bacteriology. If hospital boards of trustees understand that their hospital would not have the approval of the American College of Surgeons without this service, it would promptly be made available. Hospitals might be graded in some such way as this: hospitals equipped to perform major research, minor research, twenty-four-hour emergency service, and routine aerobic and anaerobic bacteriology would be approved and given A+; hospitals ready for all but major research would be approved with an A; hospitals prepared for twenty-four-hour emergency service and routine work would be qualifiedly approved with a B and urged to improve the service, hospitals doing only routine work would not be approved and would be rated C; and hospitals without facilities

for doing even routine aerobic and anaerobic bacteriology would not be approved and marked D.[4]

B. THE TRAINING OF SURGEONS IN BACTERIOLOGY

If there is to be any solution of these problems, it must come not from the bacteriologists but from the surgeons themselves, who in the course of their training have acquired a fundamental knowledge of bacteriology. It is difficult for a bacteriologist to attack a surgical problem, because it is almost impossible for him to acquire a surgical point of view without surgical training. Pasteur did not solve the problem of aseptic technic, but Lister, the surgeon, learned the fundamental laws of bacteriology from Pasteur and applied them to a surgical problem. The professor of surgery or the surgical chief cannot go to the professor of bacteriology and say: 'Here are our problems of infection, please solve them for us.' He must say to his internes and his staff: 'Learn the fundamentals of bacteriology and apply them to our problems of infection in surgical conditions.' A large part of the time in training to be a surgeon must be spent in acquiring the manual dexterity needed to perform operations. Similarly, a large proportion of a surgeon's time each day must be given to the actual surgical treatment of his cases. A certain technic is also necessary in bacteriological work, but this is more easily acquired, the actual problems being solved more by reading, thinking, observing, correlating, and co-ordinating than by the actual performance of experiments.

However, for the solution of the difficulties arising in any particular hospital, only general principles can be laid down. These must be adapted and applied to the peculiar situations found in different institutions. The professor of surgery, or the surgical director, must make such arrangements as the size and quality of his staff will permit, and the results will depend on the amount of time and intelligence applied to his bacteriological problems. It would seem fundamental that the surgical internes should have as part of their regular training at an early convenient period in their rotary service the responsibility for doing the routine and emergency bacteriology of the surgical department. They must have supervisory instruction from the staff bacteriologist or from one of the surgical department trained in bacteriology. The former is preferable, for the staff bacteriologist is more likely to be able to develop a broader bacteriological point of view. Furthermore, at night or on holidays, the surgical internes may be required to do the emergency bacteriology for the medical service, just as the medical internes may be called upon in emergency to make cultures from surgical specimens.

If the surgical internes are required to do the bulk of the routine and

emergency bacteriology for the surgical department, the surgical point of view will be brought to the study of the specimen. Instead of having the specimen studied in a routine and perhaps abstract way as 'pus,' it will have a more particular individuality. In such a case, the report is much more likely to be of some significance. During the period of bacteriological study and service, this work need not be the interne's only duty, but it should be paramount and should be performed as the most important duty of the day. In cases of emergency, he must be available to receive and cultivate the material as soon as possible after it is obtained from the patient. No time must be allowed for the material to dry and therefore yield nothing on the culture when viable organisms are really present in the material at the time obtained. The period of the interne's service must be long enough for him to learn all the bacteriological methods, including anaerobic as well as aerobic culture, fermentation and proteolytic reactions, special media and methods for special organisms, animal inoculation, immunological reactions, vaccine and serum preparation, complement fixation, and agglutination tests. Four months' training of this kind has been found adequate for the interne. If time permits, the interne may assist in the special studies or major research under the direction of one of the older members of the staff, and this will increase his interest in, and give significance to, his routine and emergency duties.

The special intensive study of a single interesting case can best be carried out by one of the resident or attending members of the staff who is not deluged by routine duties and can have some time to read, study, think, correlate, and apply his knowledge of fundamental bacteriology to the problem under consideration. The clinical care of the case need not be in his hands, but there are many things to be said in favor of such an arrangement The interne is able to apply the treatment indicated without delay; his hands are not tied by a contrary opinion with regard to the treatment. The patient has the advantage of an individual doctor's responsibility for his case. Any change in the patient's condition is noted early and appropriate measures taken to investigate the cause, and so on. Such special investigations should be limited to the particular case and enlarged only if they are found to lead to general principles that require the carrying on of research on a larger scale.

The major or perennial problems of surgical bacteriological research should always be under close direction of one of the older men who has had both the clinical and technical experience necessary for its solution or intelligent attack. Such studies must be correlated and carried through in a logical fashion—a part at a time, but with the part seen in its relation to the whole In these problems, the director of research may have technical help, but the results obtained by such

assistants must invariably be checked by his own observations and he must constantly correlate the various parts of the problem and hold the investigations to the main purpose.

Concerning how far such researches should go in the direction of pure science, there may be a real difference of opinion. This may depend upon the number of members of the staff available for such work. If there are many, one individual might be assigned to certain abstract phases of the problem, but it would seem to be better to leave such aspects of the question to a regular member of the bacteriology department, who might have better qualifications to carry it to a successful solution.

III. CONCLUSIONS

An adequate bacteriological service for any surgical department directs its attention to routine work, to emergency work, to special research, and to general research. This service can be obtained only when the professor of surgery or the director of the surgical service demands the necessary money and equipment from the authorities, lays out a plan for the training of internes in bacteriological technic, and builds up into his own staff those qualified to carry on minor and major research in the bacteriological problems of surgery.

After nuclei of such work have been developed in many medical centers, friendly rivalry will speed the solution of these problems, and it may be possible to correlate or subdivide the problems in some way so that the best service may be obtained with a minimum of wasted time and energy.

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CHAPTER IX

The Bacteria That Produce Surgical Infectious Diseases

By Balbina A. Johnson, B.A.

Surgical infectious diseases are those whose characteristics make them amenable to surgical therapy. These characteristics are due in a large measure to the biologic activities of the different micro-organisms that cause them, and to the anatomic site in which these activities take place. In this chapter, we shall deal briefly with certain of the morphological and cultural characteristics of the various bacteria associated with 'surgical infectious diseases.' For more detailed description of the organisms, the reader is referred to standard textbooks on bacteriology (Jordan and Burrows; Park and Williams; Topley and Wilson).

The doctrine of the transmission of disease by living agents or 'contagium vivum' is centuries old. Varro[1a] (116-26 B.C.) warned of certain tiny animals that the eye could not see but that get into the body by the nose and mouth and propagate obstinate diseases. Observations made during the great epidemics that raged throughout the Middle Ages culminated in the work of Fracastorius[1b] (1478-1553), in which he states his belief that diseases can be communicated by a living agent. Acceptance of such theories, however ingenious, waited upon the demonstration of the living agents, which in turn required the invention of instruments capable of making them visible. In 1659, Kircher,[1c] using a low-power lens, reported that he observed 'minute living worms' in the blood of plague victims, and described them as the cause of the disease, but it is more probable that this was wishful thinking and that these minute forms were red blood corpuscles. In 1675, Leeuwenhoek,[1d] who brought the microscope to a very fine point of perfection, reported in a letter to the Royal Society in London that he had seen 'animalcules' in rain water. During the next fifty years he continued these letters, and so accurately did he describe and portray the little animals that he saw in saliva, diarrheal stools, and water that the bacterial forms we know today—the cocci, rods, filaments, and spirochetes—can be easily recognized. Dobell[1e] considers Leeuwenhoek 'the first bacteriologist and the

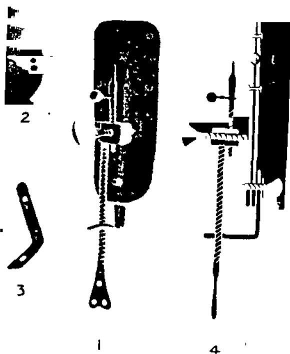


FIG. 1. Leeuwenhoek's microscope After Dobell 1932. (From Bullock's *The History of Bacteriology* Courtesy of the Oxford University Press and Dr. William Bullock)

first protozoologist who created bacteriology and protozoology out of nothing.' Although the relation of the little animals to 'contagium vivum' was far from being appreciated, the germ theory of disease continued to be propounded from time to time during the next century. In 1772, Marten [1f] published a treatise on consumption that Bulloch states 'contains nothing that is not accepted teaching on infective disease in the twentieth century.' Although this work received little attention when it was published and has been rarely referred to, Bulloch [1] believes it to be much more deserving than that of Plenciz [1g] (1762), who, although he 'expressed the conviction that living "germs" were the cause of certain diseases, contributed no new concepts or ideas.'

Still another century passed before Pasteur,[2] confirming previous work by Schwann [3-4] on fermentations, demonstrated that certain unusual fermentations in wine and beer were caused by the activity of micro-organisms. These disturbances Pasteur called 'diseases.' It was but a short step to his discovery that putrefaction as well as fermentation was produced by micro-organisms. He then proclaimed his belief that human as well as animal diseases were due to the same kind of biological process.[5] The researches of Davaine [6] (1863) on anthrax in sheep showed that it was highly probable that this disease was caused by a specific rod-shaped organism, which he called a 'bacteridium.' It remained for Koch,[7] however, to demonstrate in 1878 the complete life cycle of the anthrax bacillus. To Koch must be given the credit for placing bacteriology on a firm scientific basis. He saw the importance

of separating one bacterial species from another, and his method for the isolation of pure cultures using solid media is perhaps one of the greatest single contributions to bacteriology.[8] Koch also improved the methods of staining bacteria, and together with Weigert and Ehrlich developed the basic technical procedures used today. The method devised by Gram in 1884, who used the solutions of Ehrlich and others, is still the most widely used stain for differentiating bacteria.[9]

The period from 1876 to 1890 was in Bulloch's opinion 'the heyday of bacteriological etiological discovery.'[10] The causative agents of one disease after another were discovered by the industrious and ingenious investigations of many observers; and, although the strict etiological criterion of Henle and Koch (Koch's Postulates) could not always be fulfilled, and although some diseases baffled all methods of search, great numbers of organisms were observed and their morphological and cultural characteristics described. Attempts at an orderly classification of this large group of bacteria became imperative. Cohn, who as early as 1854 expressed the opinion that bacteria belonged to the plant rather than the animal kingdom, had persisted in his studies, and in 1872[11] became convinced that the great variety of bacterial types were constant from generation to generation He devised a classification on the assumption of unchanging morphological and biochemical properties, which although of great value in bringing order and discipline into this new science resulted in the lack of appreciation for many years of the variations that could occur in 'pure cultures' of bacteria.[12-13] Dubos[14] emphasizes that 'bacteria are more striking in their variability and plasticity than in the fixity of their morphological, biochemical, and physiological characteristics.'

Although revisions of the classification of micro-organisms are still being made as our knowledge of their properties increases, the bacteria may be divided into three major morphological groups:

1 Spherical form, the coccus
2. Rod-shaped form, the bacillus, or bacterium
3 Curved form, the vibrio, spirillum, and screw-shaped spirochete[15]

It is not possible to draw any definite conclusions about the nature of the groups of infectious agents known as 'viruses.' Some of the larger forms may differ from bacteria only in their exceedingly small size. The very minute viruses may be autocatalytic proteins lying in the borderland between the animate and the inanimate.[16]

Bacteria are further subdivided on the basis of their behavior when stained by the Gram technic. Organisms that retain the crystal violet stain are called Gram-positive, those that release it and take on the counterstain are called Gram-negative. Although when this method was

developed it was one of convenience only, it is now realized that this difference in behavior represents important and fundamental differences in cell structure.

One difference in cultural characteristics is of sufficient importance to be included at this point, since it serves to divide bacteria into two main groups. In the course of his researches on fermentation and spontaneous generation, Pasteur[17] early (1861) made the observation that certain bacteria were able to multiply only when free oxygen was excluded from the environment. The bacteria that will grow in pure culture in the presence of free oxygen or a positive oxidation-reduction potential are called aerobes, those that will develop in pure culture only when free oxygen is excluded or a negative oxidation-reduction potential has been established are called anaerobes. The qualification of 'pure culture' in these definitions is necessary, since in many instances the growth of the aerobic species will use up the free oxygen present in the medium and thereby establish the proper conditions for the growth of the anaerobes.

Most of the micro-organisms found to exist in nature are of little concern to those who are interested in bacteriology solely from the standpoint of disease processes. It is well to remember, however, that it is chiefly these other bacteria that, by completing the cycle of chemical and physical processes, make possible the continuance of life upon earth; they function in the breakdown of the complex chemical compounds, contained in the bodies of dead animals and plants, into the same chemicals that growing plants require for the synthesis of their own living tissues. These in turn are consumed by and make possible the growth of animals and man. Of almost equal importance are the anabolic activities of certain bacteria that make nitrogen available for growing plants. These organisms do not concern us at present.

Of the relatively small group of bacteria that produce pathological processes in man and animals, there is a still smaller group that will be considered here. These are of paramount interest to surgeons because of their causal or associated relationship to the so-called 'surgical' infectious diseases. These organisms will be considered in the following order.

- I. Aerobic Bacteria
  - A. Aerobic Cocci
    - 1. Gram-positive Cocci
      - a. Streptococci
      - b. Pneumococci
      - c. Staphylococci
    - 2. Gram-negative Cocci
      - a. Neisseria

B. Aerobic Bacilli
  1. Gram-positive Bacilli
    a. *Bacillus anthracis*
    b. *Bacillus subtilis*
    c. *Corynebacterium diphtheriae*
    d. *Mycobacterium tuberculosis* (Tubercle Bacillus)
  2. Gram-negative Bacilli
    a. Coli-Aerogenes Group
    b. *Eberthella typhosa*
    c. *Klebsiella pneumoniae* (Friedlander's Bacillus)
    d. Proteus
    e. *Pseudomonas aeruginosa* (Bacterium pyocyaneum)
    f. *Malleomyces mallei* (Glanders Bacillus)

II. Anaerobic Bacteria
  A. Anaerobic Cocci
    1. Gram-positive Cocci
      a. Streptococci
      b. Pneumococci
      c. Micrococci
    2. Gram-negative Cocci
      a. Neisseria
      b. Veillonella
  B. Anaerobic Bacilli
    1. Gram-positive Sporulating Bacilli (Clostridia)
      a Histotoxic or Gas-gangrene Group
        (1) *Clostridium welchii*
        (2) *Clostridium novyi*
        (3) *Clostridium septicum* (*Vibrion septique*)
        (4) *Clostridium sordellii* (*Clostridium œdematoides*)
        (5) *Clostridium sporogenes*
      b. Neurotoxic Species
        (1) *Clostridium tetani*
      c. Other Clostridia
    2 Gram-positive Nonsporulating Anaerobic Bacilli
      a. *Bacterium ramosus*
    3. Gram-negative nonsporulating Anaerobic Bacilli
      a. *Fusobacteria*
      b. Bacteroides

III. Microaerophilic Bacteria
  A. Microaerophilic Streptococci
    1. Hemolytic Streptococci
    2. Nonhemolytic Streptococci
  B. *Clostridium histolyticum*

IV. Spirochetes
   A. *Treponema pallidum*
   B. 'Commensal Spirochetes'
V. Fungi
   A. Actinomyces
   B. *Cryptococcus neoformans* (*Cryptococcus hominis, Torula histolytica, Debaromyces neoformans*)
   C. *Blastomyces dermatiditis*

I. AEROBIC BACTERIA

A. AEROBIC COCCI

1. Gram-positive Cocci

A. STREPTOCOCCI

From the standpoint of human disease, the streptococci comprise by far the most important group of bacteria, because of the frequency and severity of the infections they produce. Although Rosenbach[18] (1884) must be credited with the first clear-cut description of 'streptococcus' as a genus, it is to Billroth[19] (1868) that we owe the name streptococcus or 'berry' coccus. This group contains all the chain-forming cocci insoluble in bile. They are widely distributed on the skin and mucous membranes of man and animals and may be pathogens or saprophytes. The first successful attempt at classification of the streptococci was that of Schottmuller,[20] who in 1903 divided them into broad groups on the basis of the difference in their action on red blood cells in blood agar. Brown[21] extended this classification and defined the various gradations more precisely. The term 'alpha hemolytic' was assigned to those strains causing a partial hemolysis and a green discoloration of the red blood cells around the colony, 'beta hemolytic' to those causing a zone of complete lysis of the red blood cells surrounding the colony, and 'gamma hemolytic' to those having no effect on the adjacent red blood cells. This nomenclature has been the accepted one for the past twenty-five years. The three groups are popularly known as (1) green or greening, (2) hemolytic, and (3) indifferent, respectively. The term 'nonhemolytic' is also used to differentiate all streptococci that do not produce an extracellular soluble hemolysin from those that do—the hemolytic streptococci. This latter grouping is very satisfactory as far as surgical infection is concerned, provided we do not lose sight of the fact that the red blood cells of all species are not equally susceptible to the streptococcal hemolysins, and that the beta hemolytic streptococci may produce alpha hemolytic or green variants that may give rise to infec-

tions clinically indistinguishable from those caused by beta hemolytic strains.[22,3]

(1) *Hemolytic Streptococci:* This group, which produce an extracellular hemolysin for red blood cells, are the etiological agents in lesions within the body that differ markedly from each other not only in the anatomic site but also in clinical manifestations. These infections may involve a very small well-localized area, or they may invade practically every organ and tissue. Streptococci are usually found in the more severe abscesses and in cellulitis. They frequently invade the blood stream. They cause acute lymphangitis, lymphadenitis, suppurative tenosynovitis, and acute arthritis, and are common in puerperal sepsis and empyema. In children, they frequently produce osteomyelitis. In general, hemolytic streptococcal lesions are characterized by their rapid spread from the initial focus with a maximum of cellulitis and a minimum of necrosis, but at times an extensive gangrene is produced (see Chapter XIV).

Hemolytic streptococci may be present on the surface of the skin and on the mucous membranes of the upper respiratory tract, of the mouth, and of the intestinal canal. The strains primarily responsible for human infections have a natural habitat in the throats of normal persons[24-5] During the late winter and early spring months, the incidence increases and the streptococci are widely disseminated from this source. They probably do not live long outside the body, but when they are transferred from one human environment to another within a relatively short time, small numbers are able to produce disease.

(a) *Morphology.* Hemolytic streptococci are spherical or slightly ovoid cells, 0 2 to 1 μ in diameter They form chains of varying lengths, depending upon the type of medium, the rapidity of transfer, the rate of growth, and other cultural conditions. In broth, especially when blood or tissue is present, they may form chains containing 75 to 100 individual cocci (Fig. 2). In exudates from lesions, they frequently appear singly or in pairs, in phagocytes they may be seen in masses or tangled chains. Capsules are variable; they may be well defined and can be induced[26]

Hemolytic streptococci stain readily with aniline dyes and are Gram-positive.

(b) *Cultural Characteristics* Hemolytic streptococci grow readily in various liquid and solid culture media, especially if such media are enriched with sugar or serum. In broth, freshly isolated pathogenic strains show a granular type of growth, which may either cling to the side of the test tube or fall to the bottom as a sediment (matt variants), or they may grow diffusely (mucoid variants).

The colony form is subject to variation After 24 hours' incubation,



FIG. 2. Hemolytic streptococci from blood-broth culture, ×1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

they are usually small, round, and translucent, surrounded by a zone of clear hemolysis of the red blood cells. The shape and texture of the colony are related to the virulence of the strain and also vary with different serological groups.[27, 28]

(c) *Classification.* Although a correlation of certain biochemical characteristics, such as carbohydrate fermentations, thermal resistance, ability to reduce 1 per cent methylene blue added to milk, is still useful in the classification of nonhemolytic streptococci, these criteria have been outmoded by the serological classification of Lancefield,[29] who found that the hemolytic streptococci could be divided into definite serological groups based on the presence of a group-specific polysaccharide–'C' substance. This can be identified by a precipitin reaction.

Although the function of this group-specific polysaccharide in the metabolism of the bacterial cell has not been determined as yet, this 'C' substance serves the very useful purpose of differentiating the hemolytic streptococci that are primarily human pathogens (Group A) from those that are primarily animal pathogens or are associated with animal products, such as cheese. (Groups B through N). In man 95 per cent of the strains producing lesions belong to Group A, although certain strains of other groups—B, C, D, F, G, and K—have been isolated from severe and at times fatal infections.[27, 30-32] There is no correlation between the clinical manifestation, or anatomic site of the lesion, and the serological group.

Group-A hemolytic streptococci are further differentiated into 30 or more immunological types by two independent type-specific antigenic substances, 'M' and 'T.' The 'M' substance is an acid-soluble protein and can be identified by a precipitin reaction with homologous antiserum. It is present in matt or mucoid variants (virulent phase), and absent in the degraded glossy variants (avirulent phase), which are rarely if ever found in pathogenic processes.[27] Matt or mucoid variants produce anti-'M' precipitins and protective antibodies in immunized animals; the glossy variants do not. Lancefield[27] believes that the production of such

antibodies is an important factor in the mechanism of protection against infection by the streptococci.

It has now become apparent that the agglutinogen chiefly concerned in Griffith's[33] differentiation of the Group-A streptococci into specific antigenic types is 'T' substance. It is a cellular component of both the virulent and avirulent variants of Group-A streptococci and either variant will produce anti-'T' agglutinogens in immunized animals. Antibodies formed in the serum as a response to 'T' substance have no protective action against infection with a homologous type.

The typing of streptococci is of practical importance in tracing the source of community epidemics or of cross-infection in hospital wards and operating rooms. However, no one type can be implicated as the etiological agent of any one of the numerous clinical manifestations of streptococcal infections.[34] Likewise, there is no correlation between the type and the severity of any single infection or epidemic.

It is beyond the scope of this chapter to discuss the type-specific substances that have been identified in Groups other than A. An excellent review of this subject has been made by Lancefield.[27]

(d) *Toxin Production.* The extracellular toxins that have been studied most intensively are those elaborated by Group-A hemolytic streptococci. Since this group is primarily responsible for human infections, these will be considered briefly at this point.

1. *Hemolysin.* Todd[35] has shown that Group-A streptococci form two soluble hemolysins, an oxygen-labile hemolysin (streptolysin O), and an oxygen-stable hemolysin (streptolysin S). The oxygen labile hemolysin is antigenic, and antibodies are formed in the serum during streptococcal infections. These antibodies are the basis of the clinical 'antistreptolysin' test used to diagnose previous or suspected infections. This hemolysin is also elaborated by certain strains of Group-C and Group-G streptococci Streptolysin 'S' is specific for Group-A streptococci only.[36]

2. *Leucocidin.* The nature of this toxin, which destroys white blood cells, was reinvestigated recently by Todd,[37] who concluded that the leucocidin produced by Group-A hemolytic streptococci was identical with streptolysin 'O.' This is in accordance with the work of Channon and McLeod[38] However, in 1933 Gay and Oram[39] came to the opposite conclusion, namely, that streptococcal leucocidin was distinct from hemolysin. The possibility that these streptococci produce more than one leucocidin remains to be proved.

3 *Fibrinolysin.* Tillett and Garner[40] reported in 1933 that broth cultures of hemolytic streptococci contained a lysin that dissolved human plasma clots. It is an extracellular enzyme secreted by the living organism[41] It is antigenic in nature, and antifibrinolytic properties may be demonstrated in the serum during the course of the disease or during convalescence. Fibrinolysin is also produced by the members of Group C that are pathogenic for man, and by Group-G streptococci.[27]

4. *Erythrogenic Toxin.* This toxin was first discovered by Dick and Dick[42] in 1924, who reported that filtrates of broth cultures of hemolytic streptococci isolated from cases of scarlet fever produced a local erythema if injected intradermally. The toxin is formed by most Group-A streptococci and is used to test the immunity of individuals to scarlet fever (Dick Test), and to immunize against this disease. However, immunity to clinical scarlet fever does not mean immunity to other clinical manifestations of streptococcal infections.[43]

(e) *Other Factors Related to Virulence.*

1. *Capsular Substance.* The capsular substance in mucoid strains of Group-A and Group-C hemolytic streptococci is a nonspecific polysaccharide that has been shown to be hyaluronic acid.[44] Although it is not the only factor related to virulence, it may well be an important one. Seastone[45] found that the amount of mucoid polysaccharide present in the capsules of strains isolated from human infections could be correlated with the severity of the infection McClean[46] believes that it may act as a powerful protective agent for the organisms. Since it is a normal constituent of the tissues, it is nonantigenic and therefore cannot be attacked by the host's defense mechanism.

2. *Spreading Factor.* The 'spreading factor' described by Duran-Reynals[47,8] in 1933 increases the permeability of tissues and is thought to play a role in the invasiveness of infections. Certain strains of hemolytic streptococci contain this factor. Some workers believe that in streptococci it is identical with hyaluronidase, but this is still uncertain. Hobby et al.,[49] have reported that hyaluronidase was not found in some of the Group-A 'invasive' strains that they studied. The recent work of Crowley,[50] who found hyaluronidase in only two types (4 and 22) of the strains studied, would seem to lend weight to the view that it is not solely responsible for the invasiveness of streptococcal infections.

(f) *Pathogenicity.* The pathogenicity of hemolytic streptococci can be correlated to a large extent with the serological groupings of Lancefield,[27] although this association may be fortuitous. Group-A streptococci are primarily pathogenic for man—human pyogens. Group-B streptococci are most frequently found in bovine mastitis, and Group C in animal infections in general—animal pyogens. However, members of all Groups have been isolated from pathogenic processes of man or animals. The virulence of Group-A strains for animals is of interest because of their role in in-vivo experimentation. There is no parallel between the pathogenicity of a given strain for man and for animals. Some strains that produce a very virulent infection in man must be injected in large doses to kill mice. In general, however, mice and rabbits are susceptible to human pathogenic Group-A strains, while cats, dogs, and guinea pigs are relatively resistant.

(2) *Nonhemolytic Streptococci:* Since, in the production of surgical infections, there is little difference between the streptococci that cause a

green discoloration of blood agar and those that have no effect on the red blood cells, these streptococci will be considered as one group. They are normal inhabitants of mucous membranes and are present in the mouth, the nasal passages, the intestinal tract, and less often in the genitourinary tract. There is a difference of opinion about whether these nonhemolytic streptococci should be considered true pathogens.[51] However, there can be no doubt that they are 'opportunist pathogens,' since, when suitable conditions are present in the host, they invade tissues and cavities and produce infection. They have been assigned an etiological role in dental caries, apical abscesses, and low-grade infections of the tonsils, pharynx, and neighboring sinuses. They are frequently found in pure culture in the peritoneum following perforations of the duodenum. In perforations of the appendix, they are not uncommon in the peritoneal exudate, and they may occasionally pass through an inflamed appendix wall without perforation. They may frequently be cultured from the contents or wall of an inflamed gall bladder and share with *Escherichia coli* and *Eberthella typhosa* the responsibility for the production of infectious cholecystitis and cholelithiasis. In association with *E. coli*, they may also produce liver abscesses. Not infrequently, low-grade abscesses in the brain yield nonhemolytic streptococci in pure culture. In the common form of bacterial endocarditis, these streptococci multiply on the injured heart valves and may be cultured from the blood stream The streptococci responsible for bacterial endocarditis do not belong to any one species, but to the many varied types normally found on mucous membranes and are of both the green and indifferent varieties.[51]

(a) *Morphology.* These streptococci are spherical or ovoid, and grow in pairs, in long and in short chains. More rarely, their appearance may be almost bacillary. No capsules are formed. Motile forms have been reported. They are Gram-positive.

(b) *Cultural Characteristics.* In broth, there is abundant diffuse growth with a varying amount of deposit. On blood agar, the colonies are small, round, and translucent. They may be surrounded by a zone of greenish discoloration of the red blood cells, with at times a partial hemolysis of the blood cells immediately adjacent to the colony (alpha hemolysis), or they may have no effect on the red blood cells (gamma hemolysis).

(c) *Classification.* The biochemical characteristics of the nonhemolytic streptococci have been extensively studied in an attempt to bring about an orderly arrangement of this very heterogeneous group. Sherman[52] found that this was possible only by a correlation of certain biochemical reactions, and that consideration of any single characteristic could result only in 'utter confusion.' In Sherman's classification the non-hemolytic streptococci are distributed among three groups: (1) the viridans group, (2) the lactic group, and (3) the enterococcus group. In the viridans group

are found the human strains normally present in the mouth and upper respiratory tract; in the enterococcus group, the normal inhabitants of the intestinal canal. The choice of the term 'viridans' is perhaps unfortunate, since all of these groups contain strains that have no effect on red blood cells as well as those that cause a green discoloration. In addition, some strains of enterococci produce clear hemolysis, but these are sharply differentiated from the Beta hemolytic strains of Sherman's pyogenic group by their biochemical characteristics and virulence for man and animals. Sherman believes that without in any way disputing the usefulness of hemolytic activity in a broad classification of the streptococci, other physiological characteristics should be included in attempts to establish relationships among these organisms. With the more extensive use of serological methods it has become apparent that certain nonhemolytic strains are members of the same serological groups as the hemolytic streptococci. This may well be of epidemiological and therapeutic importance.[27] Although nonhemolytic strains of Group A are rare, green dissociants of Beta hemolytic strains were recovered by Fry,[53] Coburn and Pauli,[23] and Colebrook et al.[22] Lancefield[27] and Stableforth[54] found Group-B nonhemolytic streptococci in strains isolated from bovine mastitis, and Little[55] also found nonhemolytic Group-C strains in bovine mastitis. Sherman[52] has shown that the nonhemolytic species as well as the hemolytic species of the enterococci belong to Group D. Nonhemolytic strains are occasionally encountered in Groups G, H, and K.[56] Penistan[57] recently reported a fatal bacterial endocarditis due to a nonhemolytic Group-K streptococcus, and also a brain abscess secondary to frontal sinusitis in which the predominating organism was a Group-K nonhemolytic streptococcus. It would seem therefore that, as Lancefield[58] states, 'the correlation between hemolytic capacity and serological grouping is less strict than at first supposed.'

(d) *Toxin Production.* There is no definite proof at the present time of any filterable toxins being formed by green or indifferent streptococci. They produce neither a hemolysin nor a fibrinolysin acting on human fibrin.

(e) *Pathogenicity.* There is little doubt that in this group are strains potentially pathogenic for man as well as others that are purely saprophytic. The nonhemolytic streptococci are as a rule nonpathogenic for animals, although some strains may show a low degree of pathogenicity. They have been reported to cause arthritis and valvular lesions in the rabbit when large doses are injected intravenously.

B. PNEUMOCOCCI

It is interesting indeed that in 1881 Pasteur[59] in France and Sternberg[60] in the United States working independently published the first

scriptions of the pneumococci. Their etiological role in . established in 1880 by Fraenkel,[61-2] and by Weichsel- demonstrated their significance in human disease. Pneu- the chief and most important clinical manifestation of human n with these organisms. In a survey of over 25,000 cases during a ,ear period in six representative states Bumreich et al.[64] found that ,proximately 82 per cent of lobar pneumonias and 77 per cent of all pneumonias were caused by the pneumococcus. The surgical infections produced by this group of organisms are usually associated with either a pleural or peritoneal infection. In pneumonic lesions, these organisms may invade the pleural cavity either by direct extension or through the blood stream and infect the pleural exudate that is present in all cases of pneumonia, though to a variable extent. If suppuration takes place, *this generally becomes a surgical rather than a medical problem* The pericardium may become involved through extension from the pleura or blood stream and may also require surgical therapy. Suppurative processes in the joints at times follow invasion of the blood stream, or the infection may localize in the meninges. Surgical treatment is indicated in the former condition if it does not respond to sulfonamide or penicillin therapy. In meningitis prior to the use of sulfapyridine and penicillin, the outcome was almost invariably fatal. Now there is a high percentage of cure. Pneumococci may also cause mastoiditis, and are present in infections of the tonsils and nasal sinuses, and in lung abscesses.

The pneumococcus is a relatively common inhabitant of the mouth and may be carried there by normal persons without the production of disease. The 'carrier rates' in groups of persons examined is reported to be about 40 to 60 per cent. This percentage varies with the season of the year, showing a considerable increase in the winter months. It is also somewhat higher among 'contacts' than among 'noncontacts.' The less virulent types are more common in the normal upper respiratory tract, but Types I, II, and III are by no means infrequent.[65-6]

(1) *Morphology:* In body exudates, typical pneumococci occur in pairs surrounded by a heavy capsule, easily seen in suitably stained preparations. The individual cocci are small and somewhat elongated, one end is flame- or lancet-shaped, the other flat. They may also occur in short chains, especially in artificial media. They are nonmotile and nonspore-forming. Pneumococci are Gram-positive (see Fig. 3).

(2) *Cultural Characteristics:* These organisms grow poorly in ordinary laboratory media and require enrichment of the medium with body fluids. In serum broth, they produce a diffuse turbid growth with a slight deposit on prolonged incubation. On blood agar, the colony form is usually a small, raised, saucer-shaped disc, with a smooth glistening surface



FIG 3 Hiss capsule stain for pneumococci from blood-agar plate culture, × 1500. (From *Nelson's Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

surrounded by a zone of green discoloration of the red blood cells. These colonies are typical of the 'smooth' strains of pneumococci that are encapsulated and virulent. Reiman and others [67-9] have shown that the pneumococci may form two types of colonies, 'smooth' and 'rough.' The colonies of the rough variants are generally larger, and have a ragged periphery and a granular or uneven texture. The organisms in such colonies generally lack a capsule, are relatively avirulent, and have lost the capacity to type with specific antisera.

Pneumococci are differentiated from streptococci by their solubility in bile or pure bile salts such as sodium desoxycholate. Harris and McClure [70] and Bayliss [71] have shown that such detergents as dupanol and sodium lauryl sulfate will also lyse pneumococci.

Pneumococci lack catalase, and as broth cultures age, especially if exposed to light, they tend to sterilize themselves through the production of hydrogen peroxide. This lysis of the pneumococcal cell has also been attributed to the production of a specific autolysin.

(3) *Classification:* The antigenic structure of the pneumococcus is probably the most complex of any known bacterium and has received extensive study. The most important antigen from the medical and surgical point of view is the polysaccharide in the capsular substance. It is antigenically different in different strains of pneumococci and has resulted in the serological classification of pneumococci into the now amazing total of seventy-five specific types and subtypes.[72-3] Neufeld [74] in 1902 first noticed that specific antiserum caused the pneumococcus capsule to become greatly swollen—the 'Quelling phenomenon.' In 1901, Neufeld and Haendel [75] reported antigenically different types by serum protection in mice. Dochez and Gillespie [76] in 1913 divided the pneumococci into Types I, II, III, and a heterogeneous Group IV. Cooper et al.[77] succeeded in further differentiating this heterogeneous Group IV into twenty specific types. Since 1944, new types and subtypes have been described by several workers, making seventy-five types and subtypes that are now recognized.

The 'Quelling' reaction is used in the identification of specific types either directly from body exudates or from cultures. The specific capsular substance may also be identified by agglutination tests, precipitin tests with extracts or autolysates of pneumococcal cells, or by mouse-protection tests. Heidelberger et al.[78] investigated the chemical nature of the capsular antigenic substance and in the types studied found it to be a polysaccharide. It has a direct relationship to virulence and pathogenicity. Freshly isolated pneumococci from disease processes are usually encapsulated. Loss of type-specificity is correlated with loss of virulence and loss of demonstrable capsules.

(4) *Toxin Production:* Many attempts have been made to demonstrate a specific soluble toxin responsible for the character of the lung lesions and the severe intoxication in man, but so far results have been disappointing. Pneumococci produce an extracellular filterable hemolysin, which is inactivated by oxidation. It is antigenic in nature.[79] Human red blood cells are slightly more sensitive than those of the rabbit, sheep, and guinea pig. A leucocidin is also produced.[80] Hyaluronidase is elaborated by some strains of pneumococci. Humphrey[81] found that 56 out of 81 strains isolated from successive cases of pneumonia produced this spreading factor. There was no correlation in his series of cases between the amount of hyaluronidase produced and clinical virulence or between enzyme production and pneumococcal type. The necrosis of the skin, hemorrhagic edema of the lungs in guinea pigs, and the purpura produced in mice by injection of cell autolysates would seem to be due to an endotoxin rather than a soluble exotoxin.

The capsular polysaccharide, however, plays an undisputably important role in the host-parasite relationship. Pneumococci isolated from infectious processes are always encapsulated. Encapsulated pneumococci are much less readily ingested by phagocytes, and when ingested may even destroy the phagocytic cell. It has been demonstrated in mice that this inhibition of phagocytosis, one of the defense mechanisms of the host, is due to the activity of the capsular polysaccharide.[82] We must not lose sight of the fact, however, that the virulence of any given strain is not dependent entirely upon one or several properties of the bacterial cell. The host factors are fully as important in determining the clinical manifestations and severity of the disease.

(5) *Animal Pathogenicity:* The pneumococcus is particularly virulent for mice, slightly less so for rabbits. Guinea pigs, cats, dogs, and fowl are relatively resistant to infection with this organism. The virulence of pneumococci for mice has made possible the rapid isolation and typing of these organisms from sputum. If a small quantity of washed sputum containing virulent organisms is injected intraperitoneally into mice,

22° C. than at 37° C., and on serum than on plain agar. The pigment itself is nonantigenic, and there is no evidence that it is related to the pathogenicity of the strain.

(3) *Classification:* The differentiation of staphylococci on the basis of pigment production on solid media dates from the work of Rosenbach, published in 1884.[86] The colonies of *Staphylococcus aureus* are characterized by a rich golden pigment; those of *Staphylococcus albus* are porcelain white. It is the opinion of many bacteriologists at present that the pyogenic cocci should be considered as one group, since they represent a gradual descent from the richly pigmented, hemolytic, essentially pathogenic staphylococcus that is an active fermenter of mannitol and an active liquefier of gelatin to the nonpigmented, nonhemolytic, essentially saprophytic staphylococcus that does not ferment mannitol and may fail to liquefy gelatin. The question whether *Staphylococcus aureus* and *Staphylococcus albus* are valid species or should be considered varieties of a single species is of less interest to the surgeon than is a method for differentiating between potentially pathogenic and nonpathogenic organisms.

The cultural characteristics most frequently used in the attempt to distinguish between potentially pathogenic and nonpathogenic staphylococci by *in vitro* tests are: production of hemolysin, formation of pigment, fermentation of mannitol, and production of coagulase. From the evidence at hand, it would seem that the production of coagulase is the most reliable single criterion of potential pathogenicity,[87-91] especially since it is a constant characteristic of a given strain. Hemolysis on blood-agar plates and the formation of a golden pigment usually accompany the production of coagulase in strains isolated from pathogenic sources. However, hemolytic activity and pigment formation are characteristics that are subject to variation in a given strain. Moreover, strains that produced no demonstrable hemolysin or did not form pigment have been shown to produce lesions in experimental animals or have been recovered from pathogenic sources. The fermentation of mannitol is a valuable confirmatory test, but too great reliance cannot be placed on it as a single criterion since many nonpathogenic strains also ferment this sugar.

Good correlation is also reported between pathogenicity and the ability of staphylococci to form a specific polysaccharide, which may be identified by a precipitin reaction. The majority of pathogenic strains fall into Julianelle's Type A, and the nonpathogenic strains into Type B.[92-4]

At present, micrococci and staphylococci are classified as two different genera in the family, *Micrococcaceae*. Bergey[95] defines the micrococci as 'facultative parasites or saprophytes' and the staphylococci as 'usually parasites.' Both groups have the same general morphology. They occur

in irregular masses and may or may not form pigment. Many of the micrococci are also found on intact skin, in the sweat and sebaceous glands, and on mucous membranes of the upper respiratory tract, and the alimentary and the genital tracts as well as in the air, dust, soil, and water.

The division into micrococci and staphylococci has resulted in a lack of appreciation of the pathogenicity of certain species of micrococci. Few references are made in textbooks or in the literature to *Micrococcus aurantiacus*. In the 'Study of Accidental Contaminated Wounds' in ten hospital units throughout the United States, *M. aurantiacus* was frequently isolated from cultures of tissues débrided at the primary operation and was not uncommon in pure or mixed cultures of low-grade and chronic infections developing in such cases.[177] *Micrococcus epidermidis* is also found in infectious processes. In our experience, *M. aurantiacus* was not an infrequent coagulase producer. Coagulase positive strains of *M. epidermidis* were more uncommon. It would seem therefore that the classification of the cocci growing in irregular masses and having a single plane of division into different genera should still be regarded as tentative.

(4) *Toxin Production:* Pathogenic staphylococcal cultures contain filterable antigenic exotoxin. It is still a matter of controversy whether the factor responsible for the dermonecrotic action on subcutaneous injection, the lethal action on intravenous injection, the hemolysis of red blood cells, and the death of leukocytes represents a single toxin with different manifestations or a group of specific toxins.[67] That individual strains produce these manifestations of toxicity in different proportions would seem to prove that they are distinct.

(a) *Hemotoxin.* Glenny and Stevens and others[96-7] found that certain strains produced two hemotoxins—an alpha hemotoxin that caused rapid hemolysis of both rabbit's and sheep's cells and a beta toxin that did not lyse rabbit's cells but did lyse sheep's cells if the cultures were ice-boxed after incubation at 37° C. (hot-cold lysis). These authors report that their strains that produced alpha hemotoxin also elaborated the skin-necrotizing and lethal toxins. Filtrates containing only beta hemolysin lacked this necrotizing and lethal action. Both hemotoxins are antigenic. The antibody content of staphylococcus antitoxin is measured by the alpha antitoxin content of the immune sera.[96]

(b) *Leucocidin.* Van de Velde[98] in 1894 was the first to describe the destruction of white blood cells by staphylococcal broth cultures. Panton and Valentine[99] believe that leucocidin must be considered as distinct from alpha hemotoxin. It is produced by the majority of pathogenic staphylococci and is antigenic in nature. Antileucocidin in the serum has

been used as an index to the formation of antibodies against staphylococcal infections.

(c) *Fibrinolysin.* Some strains of pathogenic staphylococci possess the ability to liquefy plasma slowly.[100-101] It is not as constant a property of staphylococci as of hemolytic streptococci and is limited almost entirely to strains recovered from human sources.

(d) *Enterotoxin.* Certain strains of staphylococci produce an enterotoxin that has been the cause of severe outbreaks of food poisoning. This enterotoxin may be elaborated in contaminated bakery products, milk, cheese, ice cream, meat, and meat products.[102]

(e) *Spreading Factor.* Duran-Reynals[48] reported the presence of a spreading factor in certain invasive strains of staphylococci. Schwabacher et al.[103] studied 654 coagulase-positive strains of staphylococci; 93.6 per cent produced hyaluronidase, 91.1 per cent, alpha hemolysin. Of the 160 strains of coagulase-negative strains of staphylococci and micrococci they studied, they found that none produced hyaluronidase or alpha lysin. They believe that hyaluronidase production may prove to be one of the more constant characteristics of staphylococci isolated from infectious processes in man and may take its place with coagulase and alpha lysin production as a criterion of potential pathogenicity.

(f) *Coagulase.* Loeb in 1903[104] first reported the ability of broth cultures of staphylococci to coagulate blood plasma. Smith and Hale[105-6] in 1944 advanced an explanation for the association of coagulase production with pathogenicity. They found that there was a remarkable inhibition of phagocytosis of coagulase-producing staphylococci in citrated plasma. They believe that this is due to an interaction between coagulase and fibrinogen, which forms a fibrin barrier at the surface of the organism. The fibrin envelope around the cells may obstruct phagocytosis by preventing opsonification or may keep the phagocytes from reaching the bacteria by entangling them in the fibrin. There is no evidence that coagulase is antigenic. It is not limited to staphylococci, for it is occasionally produced by *E. coli,* the Pseudomonas group, and *B. subtilis* strains.

(5) *Animal Pathogenicity:* The rabbit is the most suitable of all laboratory animals for the production of experimental infections. Subcutaneous or intramuscular injections of a virulent strain produce localized abscesses; intravenous injection is usually fatal. A generalized infection results with multiple abscess formation. Intrapleural inoculation usually results in a suppurative pleurisy. Intraperitoneal injection is less successful. Osteomyelitis may be produced by injecting cultures into fractured bones. Rats, guinea pigs, and mice are much more resistant.

2. *Gram-negative Cocci*

A. NEISSERIA

(1) *Gonococci:* The activity of gonococci comes within the scope of surgery principally through errors in diagnosis. Operations are often performed in cases of acute peritonitis, suppurative tenosynovitis, or epididymitis because it is impossible to tell, before a culture of the exudate has been made, whether the infectious agent is the gonococcus or some other organism. The response of these infections in both acute and chronic forms to sulfathiazole and penicillin has taken them out of the field of surgery. Gonococci generally limit their activities to the human genital tract.

In adults the organism is transmitted in almost all cases through sexual intercourse, producing in the male a primary urethritis which frequently spreads to the prostate gland, seminal vesicles, and epididymis; and in the female a primary cervicitis which frequently spreads to the glands about the vagina, urethra, and through the cervix and body of the uterus to the Fallopian tubes. In many female cases, there is an acute inflammation of the peritoneum, which at times reaches alarming proportions but rarely causes death. In a relatively small number of cases the organism is found in suppurative processe in joints, and rarely in vegetations of the heart valves and in the meninge Its activities come within the realm of surgical therapy chiefly in its involv ment of the epididymis, the Fallopian tubes, and the joints. The gonococcus the common cause of the ophthalmia of the newborn and of the vaginitis female infants. In the former, it is agreed, the source of infection is the gen tract of the mother. Apparently the vaginitis of infants is of a mild chara and does not usually spread to neighboring organs or cause chronic infl matory processes As is the case with so many other organisms, the gono do not form a homologous group. Pearce, in 1915,[107] showed that gono from the urethra of adult males and from the vaginitis of infant females serologically different.

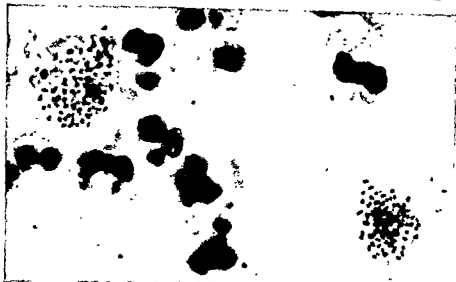


FIG. 5. Gonococci in urethral exudate are chiefly intra-cellular and seldom mixed with other organisms, × 1500.

best results are obtained if warmed proteose-3 hemoglobin agar or chocolated blood-infusion agar is inoculated immediately after the culture is taken, since these cocci die off rapidly if exposed to the air and allowed to dry. Gonococcus colonies are small and convex with an undulate margin. They have a pearly opalescence when viewed by transmitted light. They grow best in an atmosphere of 10 per cent carbon dioxide. Under anaerobic conditions, little or no growth occurs. Gonococci produce an oxidase, and this property is used in the identification and isolation of gonococcus colonies in mixed cultures. When tetramethyl-p-phenylenediamine is poured or sprayed on the plate, the gonococcus colonies turn a purple color.[109] This oxidase is also formed by the meningococcus and other Neisseria. However, the gonococcus may be differentiated from the meningococcus by its failure to ferment maltose and from the saprophytic Neisseria by its failure to grow on plain agar.[110]

(c) *Toxin Production.*

The gonococcus does not produce a true soluble toxin, but extraction of the bacterial bodies results in the recovery of a toxic substance which is probably in the nature of an endotoxin. Within the body, the gonococcus rarely gives any evidence of a severe intoxication, except in widespread infections of the peritoneum or in septicemia.[108]

(d) *Animal Pathogenicity.* Infection with the gonococcus occurs spontaneously only in man. It has not proved possible to produce gonorrhea in any animal, not even in the anthropoid ape. However, large doses of gonococci injected intravenously or intraperitoneally into mice, guinea

pigs, or rabbits may cause a fatal septicemia, as a result of toxic action of the organisms.[111]

(2) *Other Gram-negative Cocci:* In mixed infections of the mouth and jaw, *Neisseria catarrhalis, Pharyngis sicca,* and so on may be present as part of the bacterial flora. They do not play a pathogenic role in surgical infections.

B. AEROBIC BACILLI

1. *Gram-positive Bacilli*

A. BACILLUS ANTHRACIS

This organism is of considerable interest historically because it was the first to be definitely proved to be the cause of disease.[6] Anthrax is very prevalent, particularly in sheep and cattle, and geographically is the most widespread of all infectious disorders; it formerly ravaged the herds of Europe and Asia, at times to an alarming degree.[112] The disease occurs in man chiefly among those who care for these animals or who handle their hides. Several epidemics have been traced back to shaving brushes that carried the organisms in the hair. From the surgical point of view, infection with this organism is chiefly of interest because of the production of furuncles of the skin and abscesses of the subcutaneous tissue that may be difficult to differentiate from lesions produced by the streptococcus and staphylococcus. If the infection takes place from the outside, a local process develops, which has been given the popular name of malignant pustule, because of the severity of its local manifestations Frequently, milder lesions are produced indistinguishable from the ordinary boil or carbuncle except by microscopic examination of the pus or by cultivation of the organism.

(1) *Morphology Bacillus anthracis* is a large, nonmotile rod, varying considerably in length. In general, the organisms appear in long chains or segmented threads, each unit with ends cut off abruptly. The organism is surrounded by a capsule. When stained by Gram's method, it retains the violet. Spores are formed, which are centrally placed. The spores may remain alive and retain their virulence for as long as twenty years (see Fig. 6).

(2) *Cultural Characteristics:* The organisms grow readily in the ordinary laboratory media. On agar plates they develop an irregular colony, which later spreads out, showing under the microscope characteristic tangled threads. On gelatin plates, these colonies liquefy the gelatin and float on the top of the liquid medium as an irregular, white pellicle.

(3) *Toxin Production·* Neither a true, secretory toxin nor an endotoxin has been demonstrated for the anthrax bacillus, but it is quite possible



FIG 6 Anthrax bacilli and spores from agar slant, 4 days old, × 1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

that within the body it produces a toxin; for in both man and animals there is definite evidence of general intoxication. Because of its invasiveness and its tendency to multiply in great numbers within the body, it has been thought that it might have some deleterious effect by the mechanical blockage of capillaries with tangled threads.[113]

(4) *Animal Pathogenicity:* Animals differ greatly in their susceptibility to this organism. Sheep are particularly susceptible, although certain varieties show a high resistance. Dogs and cats are resistant. Man is relatively susceptible.

B. BACILLUS SUBTILIS

This organism is of interest surgically because of its appearance in operative wound infections. It is a common air organism and may contaminate the wound, but probably does not of itself often produce disease. It is frequently found in association with other organisms and may have some symbiotic action with them. Cultures from postoperative hematomata may yield the organism.

(1) *Morphology: Bacillus subtilis* is a large rod, occurring singly or in chains. It is actively motile. Spores are numerous and are slightly excentric. It is Gram-positive.

(2) *Cultural Characteristics.* The organism grows readily on the simplest media. On blood-agar plates it produces a profuse growth that tends to form a dry, scaly mass. There is often a considerable degree of hemolysis, which is particularly evident when the cultures are grown under anaerobic conditions.

(3) *Toxin Production: Bacillus subtilis* does not produce toxic substances except for a hemolysin, which is relatively unimportant.

(4) *Pathogenicity:* So far as is known, *B. subtilis* is nonpathogenic, but it is not infrequently the only organism found in mild operative wound infections.

(5) *Antibiotic Production:* Several strains of this organism have been found to be capable of producing potent antibiotics, including bacitracin, subtilin, and eumycin (see Chapter XIV).

C. CORYNEBACTERIUM DIPHTHERIAE [103]

Although this organism primarily causes an infection of the throat, larynx, and nose, it has frequently been found in wounds. Such cases have been reported from time to time in the American literature, but the best review of the conditions observed is by Walter Grossmann,[114] who made a study of 400 open wounds. In 5 per cent of these wounds, true diphtheria bacilli were found. Grossmann says that it is impossible to diagnose these cases clinically because there is nothing characteristic about them, and he does not think that these organisms interfere with wound healing. On the other hand, Hoffman, Donges, and Elfeld,[115] and others, have stated that at some stage these wounds have a characteristic appearance and that the organisms may not only inhibit wound healing but may also be carried from this source to the throat. Chronic stagnant ulcers infected with these organisms have been reported, which have healed promptly following the administration of diphtheria antitoxin. In most cases, however, the diphtheria bacillus is not found alone in these infections, and the chronicity of the lesions may be due to a synergistic action of the various species present.

(1) *Morphology: Corynebacterium diphtheriae* is a Gram-positive bacillus varying considerably in size and shape. Even in young cultures it shows spindle- or club-shaped forms that, with other bacilli, are considered degenerative or involution forms but with this organism are believed to be normal, viable forms. The bacilli appear singly. They stain unevenly, as is brought out by special stains, particularly the method of Neisser, which stains ovoid bodies at the ends of the bacilli.

(2) *Cultural Characteristics:* The most important of its cultural characteristics is the fact that *C. diphtheriae* outgrows other organisms when planted on Loeffler's coagulated blood serum. After eight to twelve hours pearl-gray colonies appear, and the characteristic forms dominate the picture when the smear is stained with Loeffler's methylene blue or the Neisser stain.

(3) *Toxin Production:* Under favorable conditions all strains produce a true, soluble toxin, but strains differ markedly in the amount they can manufacture, particularly in a less favorable medium. This toxin can be effectively neutralized by the prompt administration of antitoxin, but if union has taken place between the toxin and vulnerable cells, antitoxin can no longer unite with the toxin, nor can it restore the damaged cells.

(4) *Animal Pathogenicity:* There are many strains of *C. diphtheriae* entirely without pathogenic power for animals. These are frequently found in routine examinations of normal throats, and many of Grossmann's wound cultures yielded them. Likewise, virulent strains have been found in normal throats. On the other hand, the pathogenicity of any given strain runs quite parallel for man and laboratory animals, especially rabbits and guinea pigs, and efforts have been made to make rapid determination of virulence when positive cultures have been obtained from human cases. The organism only rarely invades the body. It kills by producing toxin locally, which is absorbed and has a specific action on the central nervous system, producing paralysis and, to some extent, degenerative changes in the liver, kidneys, and adrenals.[116]

D. MYCOBACTERIUM TUBERCULOSIS (TUBERCLE BACILLUS)

From a surgical standpoint, the tubercle bacillus is by far the most important of the aerobic bacilli. The widespread manifestations of the disease processes produced by this organism have been well known for centuries, and for over two thousand years the pulmonary type has been considered a communicable disease.[117] There is no land in which tuberculosis does not exist, and in certain parts of the world, almost all persons are victims of the disease. Naegeli's statistics, quoted by Zinsser and Tyzzer,[118] based upon a large series of autopsies, showed that there was a gradual increase of incidence of active tuberculosis from a few months after birth up to the thirtieth year, when almost all cases examined showed some trace of tuberculous infection. After the age of thirty, active lesions gradually diminished, whereas healed lesions increased. This incidence undoubtedly varies according to the sanitation and the general health of the community. The incidence of tuberculosis in the United States has materially decreased in the last half-century, but it is still a tremendous factor in national morbidity and mortality.[119]

In the individual, tuberculosis may be limited to a small portion of the body, but in many cases it is extensive. There is apparently no tissue of the body immune to infection, although the most common sites are the lungs and the regional lymph glands.

As far as man is concerned, there are two main groups of tubercle bacilli that have shown distinct characteristics both in their clinical and their cultural activities. These are called the human and the bovine types. A third type, which exists in birds, need not concern us here.

(1) *Human Tubercle Bacillus.* This organism produces disease in man that varies greatly in its extent and severity. In general, the lesions are chronic, developing slowly and lasting for a long time without great evidence of a general illness. There seems to be a tendency in certain

individuals for the infection to remain in one type of tissue. For instance, in many cases, the infection is limited to the lungs; in others it seems to be limited to the lymphatic glands; in others, the skin; in still others, the bones. This fact has led to the general belief that strains differ somewhat in their predilection for certain tissues, and efforts have been made to demonstrate certain biologic characteristics corresponding to these clinical groups, but the same difficulties have been found as with other organisms, and the results have not been satisfactory. The original anatomic site of infection may determine to some extent the character of further spread of the infection. The organisms are found in the discharges of patients suffering from the disease—in the sputum, in the feces, in the urine, in discharge from the surface of skin lesions, and to a less degree in the discharge from chronic tuberculous sinuses. Because of their resistance to unfavorable environmental conditions, the organisms may live for some time outside the body, but it is believed that in most cases of infection there is a transfer of the organisms directly from individual to individual.

(a) *Morphology*. Tubercle bacilli appear as slender rods, 2.0 to 4.0 μ in length and 0.2 to 0.5 μ in width. The rods may be straight or slightly curved and frequently have a beaded appearance from an uneven absorption of the stain. Occasionally branched forms are seen, and for that reason they are grouped with the higher bacteria. The organisms are surrounded by a capsule containing a waxy substance, which protects them against drying and also interferes with the entrance of stains. Because of this waxy capsule, staining must be done with an intensely penetrating solution followed by decolorization, which deprives all but the acid-fast organisms of their color. The Ziehl-Neelsen method is now in general use. With this, the organisms appear pink, while the general background is blue (Fig. 7). When stained by Gram's method, tubercle bacilli are found to retain the dye but stain irregularly.

(b) *Cultural Characteristics*. The tubercule bacillus is difficult to cultivate because of its slowness of growth and because of its frequent association with other organisms that outgrow it on the usual culture media. Efforts have been made to destroy these concurrent organisms. In this effort Petroff,[120] who has been able to obtain pure cultures of the tubercle bacillus from sputum and feces, has been somewhat successful. Modifications of Petroff's technic have shortened the time required for the development of actively growing cultures.[121-2] Once isolated, the bacilli are best grown on glycerin-egg medium. They appear after six to eight days as yellowish-white, moist, crustlike flakes. In glycerin bouillon the bacilli as they grow spread out over the surface as a thin, opaque, floating membrane, which gradually becomes thickened and wrinkled; later, portions of the membrane sink to the bottom. The tubercle bacillus

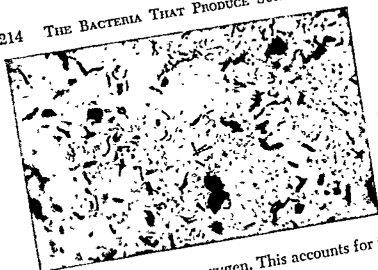


FIG. 7. Tubercle bacilli in sputum. An unusually heavily loaded sputum showing acid-fast bacilli, × 900, stained with Ziehl-Nielson's Carbol-fuchsin.

requires free access to oxygen. This accounts for its preference for surface growth.

Tubercle bacilli are more resistant to heat than most other nonspore-forming organisms. This is attributed to the protective qualities of the waxy capsular membrane. For this reason, also, the organisms stand drying and have been found viable and virulent in dried sputum for more than two months; in rooms protected from abundant light they have been found in dried sputum as long as ten months. They do not multiply in nature outside the living animal body.[123]

(c) *Toxin Production.* The tubercle bacillus produces no true toxins. Filtrates from broth cultures and extracts of the bacilli are highly toxic for tuberculous animals, producing fever and local and focal signs of inflammation. The lesion of tuberculosis is characterized by necrosis of the tissue, with a cellular reaction forming a typical microscopic picture that is used for the diagnosis of the disease even without the demonstration of the bacilli themselves These lesions may be produced by injecting dead as well as living bacilli.[124] They are called tubercles.

(d) *Animal Pathogenicity.* The tubercle bacillus is pathogenic not only for man but for the cow, monkey, pig, and cat. Young guinea pigs are especially susceptible and have been used with great success in the isolation of a small number of tubercle bacilli in suspected material when no organisms can be seen in the smear and when artificial culture is impossible. Infection is most readily produced in guinea pigs by intraperitoneal injection. If a large dose is given, death may result in ten to twenty days, at which time tubercles may be found in many parts of the body but particularly in the peritoneum and the retroperitoneal lymphatic glands. When smaller doses are given, death may not result for four to six weeks. The general routine in laboratories where this method of isolation is used is to kill the guinea pig at the end of six weeks and pronounce the test negative if there is no evidence of tuberculosis at that time. For more rapid isolation, the guinea pig may be



Fig. 8 Guinea pig killed by tuberculous infection The animal died in the sixth week following the injection of a small quantity of chest fluid Note the tubercles in (A) lung, (B) liver, (C) spleen, (D) peritoneum, and (E) abdominal lymph gland (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

injected in the thigh; if neighboring lymphatic glands enlarge, these may be examined and the organisms may be demonstrated in smears made from crushed glands (see Fig. 8).

(2) *Bovine Tubercle Bacillus.* The chief surgical interest in the bovine tubercle bacillus lies in the fact that the proportions of the human and bovine types in a large series of cases vary considerably for different anatomic lesions and for different ages. Park[125] has tabulated a series of 478 cases illustrating these points. The striking facts brought out by Park's table are: first that for adults over sixteen years of age, the bovine organism occurred in less than 1 per cent. From five to sixteen years of age, the human type predominated approximately 5 to 1, with the largest percentage of the bovine type in cases of cervical adenitis. In children under five years of age the human predominated over the bovine bacillus less than 4 to 1. In the cervical adenitis cases at this age, the bovine type occurred twice as often as the human type.

The supposition is that the greater incidence of the bovine type in children is due to milk, which in many places is not under strict supervision. It has been found that in those localities where the milk supply is under the best control and the dairies are under the strictest regulations with regard to the eradication of tuberculous animals from the herds, the incidence of bovine tuberculosis in children is markedly less than where there is no such control.

(a) *Morphology.* Zinsser and Tyzzer[126] have summarized the difference between bovine and human tubercle bacilli as follows:

> Morphologically the bovine bacillus is a little plumper and thicker than the human type On glycerin broth, the final reaction in the case of human bacilli is considerably acid, the final reaction with the bovine is very slightly above the neutral point. The bovine bacillus does not grow as readily as the human and is not aided by the addition of glycerin to the media to the same extent as is the human. The growth of the human bacillus is apt to be more luxuriant than that of the bovine, especially in earlier generations.
>
> As to virulence, the bovine is much more virulent for all of the ordinary laboratory animals than is the human.

This is particularly true in rabbits, and this fact may be used for differentiation.

2. *Gram-negative Bacilli*

A. COLI-AEROGENES GROUP

This represents a large group of organisms found almost universally in the intestinal tract of man and animals, making their appearance in the stools shortly after birth. Many attempts at classification by biologic

characteristics have been made, but the coliform group consists of a gradation of types that are closely linked, making it difficult to separate them into well-defined genera.[127-8] Although two different types—coli and aerogenes—can be distinguished by a series of biochemical tests, many 'intermediate forms' occur. Topley and Wilson[129] have pointed out that there is an important correlation between these biochemical tests and natural habitat. The *Aerobacter aerogenes* type, which was frequently isolated from soil and grass, is a much less common inhabitant of the intestinal tract.

Outside the intestinal tract *E. coli* may produce serious lesions in man, varying in character from local abscesses around the alimentary tract or local infections in the urinary bladder, gall bladder, and liver, to a general invasion of the blood, a meningitis, or an endocarditis. *Escherichia coli* is of interest to the surgeon chiefly because of its association with appendicitis and peritonitis. It may not be the primary cause of appendicitis, but when that organ becomes inflamed, *E. coli* may pass through a grossly intact wall, produce peritonitis, and be recovered from the exudate in pure culture.[130] When the intestinal wall is perforated by an ulcer or by a foreign body, *E. coli* plays a major role in any subsequent infection. *Escherichia coli* reaches the gall bladder either from the liver via the bile, or by passing up the duct. It may precede or follow the development of stones, but it shares with *E. typhosa* and the nonhemolytic streptococcus the infective role in the development of cholecystitis.[131]

In many cases of perforation of the gut with abscess formation, the colon bacillus has been considered to be the chief organism responsible for the infection, because in culture media, and even in abscess cavities, it generally outgrows other forms. It is probable that its pathogenicity in pure culture is very much less pronounced than when it is mixed with other intestinal organisms with which it normally grows in some sort of symbiotic relationship. This fact has been clearly shown in the work of Meleney and his associates.[130, 132] In a series of 106 cases of peritonitis, this organism was found in pure culture in 8 cases, all of which survived. In none of these cases was there perforation of the gut. Welch[133] likewise reported a case in which *E. coli* was found in the peritoneal fluid without any evidence of intestinal perforation. In man, bloodstream infection has been found not infrequently just before death. In newborn infants hemorrhagic septicemia, or Winckel's disease, may result from a generalized colon bacillus infection.[134]

(1) *Morphology* *Escherichia coli* varies considerably in size and shape. The usual form is a small bacillus with rounded ends, with the length two or three times the width, but many small coccoid forms and longer



FIG. 9 *E. coli* in broth culture, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

rods appear. They usually remain single but may occur in pairs or short chains. They may be motile or nonmotile. They are not usually encapsulated. They are decolorized when stained by Gram's method (see Fig. 9.)

(2) *Cultural Characteristics: Escherichia coli* grows readily on the simplest media. In broth, growth is diffuse; on blood-agar plates they form large, round, translucent colonies measuring from 2 to 3 mm. when isolated. A considerable number of strains are hemolytic. When they are grown under anaerobic conditions, the hemolytic action may be enhanced although the growth of the colony is considerably inhibited. Perhaps the most important cultural characteristics are those that distinguish this bacillus from the more virulent organisms of similar form, namely the typhoid and dysentery bacilli. The organisms of typhoid and dysentery do not ferment lactose, while the colon organisms do.

(3) *Toxin Production: Escherichia coli* does not produce a true toxin. The intoxication caused by the injection of these organisms is just as great with dead as with living organisms and is considered to be a product of dissolution rather than any specific poisonous substance within the organism itself. Some strains produce a filterable hemolysin.

(4) *Animal Pathogenicity:* The colon bacilli have little pathogenicity for animals when injected in pure culture, large quantities being borne without any reaction or with only a local development of relatively innocuous abscesses. Occasionally, however, intraperitoneal or intravenous injections will kill small animals. With certain strains the minimal lethal dose by peritoneal injection may be lowered, by repeated animal passage, to a thousand individual micro-organisms for a guinea pig or a mouse. *Aerobacter aerogenes* is usually less pathogenic for man than is *E. coli.*

B. EBERTHELLA TYPHOSA [108]

The disease produced by this organism comes within the scope of surgery, particularly in certain of its complications, the chief of these being perforation of the intestine and inflammatory lesions of the gall

bladder; more rarely, suppurative processes beneath the periosteum of the long bones; still more rarely, subcutaneous or intramuscular abscesses.[135] The complication of perforation produces a condition similar in every way to a perforation of the lower ileum from any other cause, either external or internal, the peritonitis that develops rarely being caused by the typhoid organism but most often by one or more of the intestinal organisms that escape into the peritoneal cavity. On the other hand, lesions in the gall bladder and in the bone are apparently caused specifically by the typhoid bacillus, for this organism may be obtained in pure culture from the gall bladder, or the bile, or even from the center of gallstones. In certain cases of pneumonia, empyema, and meningitis associated with typhoid fever, typhoid bacilli have been found in pure culture; but in many of the complications of the general disease, other organisms frequently play an important role. Not infrequently, positive cultures of the typhoid bacillus have been obtained many years after the active disease, and there has been considerable speculation with regard to the length of time the organism may remain within the body either in a dormant state or multiplying in the usual way without the production of illness. Martin[136] has observed a case in which the typhoid organism was obtained in pure culture from one of the long bones thirty years after the occurrence of typhoid fever. Whipple[137] has found it in a gall bladder forty-three years after the attack of fever.

(1) *Morphology:* The typhoid organisms resemble the colon group, but in general are somewhat longer and more slender. They are more actively motile, possessing many long flagella. They are Gram-negative (see Fig 10).

(2) *Cultural Characteristics:* The typhoid organisms grow readily on the ordinary laboratory media, but somewhat less luxuriantly than does the colon bacillus. On agar, the colony is smaller and the differences between the typhoid colonies and other members of the colon group in their fermentation of lactose have made possible the isolation of this organism from mixtures. With the use of lactose and an indicator in agar plates, the absence of any color change in the individual colony can readily be seen; and subsequent generations from this colony may be made for further identification in a series of sugar media. For further confirmation, agglutination tests are required.

(3) *Toxin Production:* Many workers who have studied this problem for the most part confirm the original findings of Pfeiffer (1894),[138] who maintained that poisonous substances were set free only when the

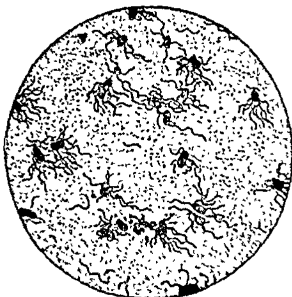


FIG. 10. *Eberthella typhosa*, stained for flagella. (From Fraenkel and Pfeiffer, *Mikrophotographischer Atlas der Bacterienkunde*, Berlin, J. Springer; and Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

bacteria were destroyed or that the poison came from a split product after their dissolution. Morgan[139-140] found that the intracutaneous injection of the toxic somatic antigen causes local edema and necrosis. Intravenous injections may cause pathology similar to that in human typhoid fever.

(4) *Animal Pathogenicity:* The typical disease as seen in man does not appear when animals are inoculated with typhoid organisms, except in the case of the chimpanzee, and it does not occur spontaneously. The organisms probably do not multiply to any great extent within the bodies of experimental animals, but injection with either dead or living organisms causes some evidence of intoxication. When live typhoid organisms are injected intravenously into rabbits, they may be recovered from the bile and may remain in the gall bladder for weeks.[141]

C. KLEBSIELLA PNEUMONIAE (FRIEDLÄNDER'S BACILLUS)

The Friedländer bacillus was originally believed to be a frequent cause of lobar pneumonia.[142] However, it has now been established that primary pneumonia due to this organism is extremely rare. Rumreich[64] reports its incidence as 0.17 per cent for both types of pneumonia in the 25,802 cases he collected. Solomon[143] gives its incidence as 0.6 per cent in the 5000 cases he reviewed. The mortality rate is high—in some series 90 per cent of the cases were fatal. The Friedländer bacillus has also been isolated from infections following perforations of the intestinal tract, and from infections of the biliary, urinary, and genital tracts as well as from upper respiratory tract infections. It may invade the blood stream, and the septicemias are often fatal. Gay

reports only 3 recoveries out of 50 cases in the literature.[144] Baehr et al.[145] report 4 recoveries in their series of 16 cases at the Mt. Sinai Hospital, New York City.

The Friedlander bacillus is a normal inhabitant of the upper respiratory and intestinal tracts of man. Although chiefly parasitic it has been recovered from air, dust, soil, and water.[146]

(1) *Morphology:* On direct examination of infectious material, this organism appears as a plump bacillus, almost coccoid in form, surrounded by a heavy capsule. When cultured *in vitro* they are short plump rods 0.5 to 0.6 μ in width, and 1 to 2 μ in length. Occasional long rods or even filamentous forms may be observed after prolonged cultivation. In fresh material, the capsule may be seen in preparations stained with carbol-fuchsin or methylene blue, but for material from cultures the Hiss capsule stain is recommended. This organism is nonmotile and produces no spores. It is Gram-negative (see Fig. 11).

(2) *Cultural Characteristics.* The Friedlander bacillus grows readily in ordinary laboratory media. In broth, there is heavy growth with the formation of mucus and a surface pellicle. On solid media typical colonies of virulent strains are gumdrop or nailhead in shape and mucoid in consistency. They string out when drawn up on a wire needle. 'Rough' variants may occur, and the organisms in these degraded forms lack the capsule and are avirulent.

(3) *Classification:* It is the opinion of many bacteriologists that the Friedlander bacillus should be considered a member of the coliform group of organisms. There are at present no valid criteria for the differentiation of this bacillus from the encapsulated Aerobacter and Escherichia, which occasionally occur.[128] The Friedlander bacilli vary considerably in their fermentation and other biochemical characteristics, and attempts to classify these organisms on the basis of such reactions are of little use. Julianelle succeeded in classifying them serologically on the basis of type-specific capsular polysaccharide into



FIG 11 *Klebsiella pneumoniae* from blood-agar plate culture, Hiss capsule stain, × 1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

Types A, B, C, and a heterogenous Group X. The majority of strains isolated from human infections belong to Type A, although the other types may also be pathogenic for man.[147-9]

(4) *Toxin Production:* Filtered cultures contain a toxin fatal to rabbits producing symptoms of paralysis. It is not certain that this is a true toxin or specific for this organism only. In general, the toxic products are considered to be due to an endotoxin. In man, when infection is once established, the organism shows evidence of great toxicity, which may be responsible in a large measure for the fatal outcome of many cases.

(5) *Animal Pathogenicity:* The pathogenicity of the Friedlander bacillus for man has been mentioned above. In laboratory experiments types A and B are highly pathogenic for mice, less so for rabbits and guinea pigs. Type C is relatively avirulent. Spontaneous infection in mice, guinea pigs, moose, and horses has been reported.[150]

D. PROTEUS

These bacilli represent a large group of organisms associated with putrefactive processes. One species, *Proteus vulgaris,* is of surgical significance. It is one of the most common organisms in soil and water and is found in sewage. It is frequently present in infections associated with the gastrointestinal tract. It has also been reported as the sole etiological agent in a number of infectious processes, but the difficulty of isolating other organisms from Proteus cultures leads one to suspect that some of these cases at least have been mixed infections. However, where the Proteus organism has been obtained from the blood stream, there seems to be definite evidence that it can be pathogenic (see Case IV in Chapter XIII).

(1) *Morphology:* These bacilli are straight or slightly curved rods, arranged singly, in pairs, or in short chains. In young swarming cultures, long filaments may occur. It is actively motile. No capsules are present. It is Gram-negative.

(2) *Cultural Characteristics:* Proteus grows readily in the simplest media, producing a thick mucoid growth with a surface pellicle. On blood-agar plates, it spreads rapidly in waves from the site of implantation and covers the whole plate with a thick film. For this reason, it is difficult to isolate other colony forms when they are associated with this bacillus unless they vary from it in heat resistance or in their reaction to some other bactericidal or inhibiting agent such as sodium azide. These organisms are actively proteolytic and produce hydrogen sulfide. The odor of a Proteus culture is characteristic and offensive.

(3) *Toxin Production:* Because of its proteolytic action, Proteus has been implicated in food poisoning. Such action imparts a disagreeable odor and taste to the food that are absent in many of the other more serious food poisonings as, for example, those due to *Clostridium botulinus.* Bengston[151] reported that a toxic product obtained from Proteus cultures produced diarrhea and vomiting when injected into dogs and rabbits.

(4) *Animal Pathogenicity:* In pure culture, the bacillus is pathogenic for rabbits, rats, and guinea pigs, if large doses are injected. Local abscesses may form, and death occasionally occurs as a result of septicemia.

E. PSEUDOMONAS AERUGINOSA (BACTERIUM PYOCYANEUM)[108]

This organism is of interest to the surgeon chiefly because of its frequency as a contaminant of surgical wounds. Cross-infections often occur in hospital wards. The bacillus frequently develops on open wounds, and, in association with the primary organism, it may at times considerably complicate and delay wound healing, and prevent the 'take' of skin grafts. Only rarely is it found to produce evidence of pathogenicity. However, such cases have been reported by Brill and Libman.[152] Recently, in the wards of the Presbyterian Hospital, New York, *Ps. pyocyanea* appeared in a wound following a cystotomy, and the organism was cultured from the blood stream. It subsequently disappeared and the patient recovered. Such infections generally occur in debilitated individuals. It has also been recovered from cases of chronic otitis media, meningitis, and cerebral abscesses. Ghosh[153] and Weidenmuller[154] have reported severe gastrointestinal disorders in adults and children.

(1) *Morphology:* It is a small Gram-negative bacillus appearing singly or in short chains. It possesses flagella and is actively motile.

(2) *Cultural Characteristics:* The chief characteristic of its growth is the production of a green pigment, which is formed in all types of media. It grows profusely on blood-agar plates under aerobic conditions, but growth is almost completely inhibited by anaerobic conditions.

(3) *Toxin Production. Pseudomonas pyocyanea* produces a toxin of mild strength with all the characteristics of a true exotoxin.

(4) *Animal Pathogenicity:* Laboratory animals are susceptible, particularly guinea pigs.

F. MALLEOMYCES MALLEI (GLANDERS BACILLUS)[108]

Although *Malleomyces mallei* primarily infects horses and mules, it not infrequently produces disease in man. It is almost never found in human beings except those who have come in contact with these animals

In man, it occurs in local lesions of the skin and subcutaneous tissue, which first appear as papules and then as pustules not unlike smallpox vaccine lesions in appearance. It becomes of interest to surgeons because of its frequent confusion with streptococcus and staphylococcus infections of a simpler nature. The condition may be exceedingly chronic, lasting for months, or very virulent, producing death within a few days A chronic form is also occasionally seen in which there are no primary skin lesions and in which the infection probably takes place through the respiratory tract.

(1) *Morphology: Malleomyces mallei* is a small rod showing considerable variations in size. It is nonmotile. It is decolorized by Gram's method

(2) *Cultural Characteristics:* Because of its frequent association with other organisms that outgrow it in the ordinary media, it is difficult to obtain this bacterium in pure culture except by animal inoculation Intraperitoneal injections in male guinea pigs produce, in two to five days, a swelling of the testicles with a central necrosis, from which pure cultures of the organism may be obtained in 50 per cent of the cases.[155] It may then be grown very easily on potato or on potato-glycerin blood agar. The growth on cooked potato is especially characteristic. A moist, transparent, yellow layer develops, which later becomes deeper in color, finally turning reddish brown.

(3) *Toxin Production.* This organism does not produce a true toxin, but the poison liberated on its death is moderately toxic.

(4) *Animal Pathogenicity: Malleomyces mallei* is very pathogenic for guinea pigs, cats and dogs, and, to a lesser degree, for rabbits and white mice.

II. ANAEROBIC BACTERIA

Pasteur in 1861 was the first to observe that certain bacteria would not multiply at atmospheric oxygen pressure, and it is to him that we owe the terms 'anerobes' and 'aerobes.'[17] In the years following Pasteur's discovery, many such organisms were reported, but it was not until World War I focused the attention of bacteriologists upon this group of bacteria that a serious attempt was made to study and classify them in a systematic fashion. The large number of wounds into which such organisms were carried from the highly cultivated soils and filth of trench warfare afforded the opportunity of studying the various anaerobic bacteria, both pathogenic and nonpathogenic. Since this time, the work of many expert observers has developed methods making the cultivation of these organisms almost as simple as that of aerobic organisms (see Chapter x). Through the use of these technics, the

biologic characteristics of the anaerobic organisms and their relation to one another have been carefully studied. The conflicting cultural characteristics assigned to many species in the older reports are probably due to the fact that, owing to inadequate methods of isolation, the cultures of the organisms described contained more than a single species.

The phenomenon of 'anaerobiosis' has been studied by a number of investigators, and various theories have been set forth to answer the question: 'Why do various organisms differ in their oxygen requirements for *in vitro* cultivation?' Among the various species of bacteria are found those with all grades of oxygen tolerance and intolerance—from those that grow only in the presence of high percentages of oxygen to those that grow only when the oxygen tension of the atmosphere is low. Pasteur[156] believed that oxygen was of itself a lethal poison to the so-called anaerobic bacteria. McLeod and Gordon[157] advanced the theory that since these organisms were deficient in peroxidase and catalase, the hydrogen peroxide formed as a result of metabolic processes in the presence of atmospheric oxygen was highly bactericidal to the peroxide-sensitive cells. However, there is no proof that these organisms form a peroxide, and this theory is no longer accepted.

Quastel and Stephenson[158] reported in 1926 that *Clostridium sporogenes* could live and multiply in the presence of free oxygen providing there was sufficient reducing substance in the medium, that is, that it was a question of the oxidation-reduction potential of the medium rather than the amount of free oxygen that determined their ability to live and multiply. No theory has been advanced as yet that explains all the observations that have been made, but the importance of the proper oxidation-reduction potential in the medium is the one that receives the major consideration. The present criterion for a true anaerobe is its ability to develop repeatedly as a surface growth in media surrounded by an atmosphere of lowered oxygen tension and its inability to develop as a surface growth under atmospheric oxygen tension.

Intermediate between the anaerobic and aerobic bacteria is another group, comprising the so-called 'microaerophilic' bacteria. These organisms grow best in a medium with a low oxygen tension, but they may grow poorly at normal atmospheric oxygen pressure upon direct isolation from the animal body, or may, after several generations on laboratory media, adjust themselves to the presence of relatively high amounts of free oxygen in their environment, and grow under aerobic conditions.

A. ANAEROBIC COCCI

1. *Gram-positive Cocci*

A. STREPTOCOCCI

(1) *Hemolytic Streptococci:* Although a considerable proportion of strains of hemolytic streptococci grow on the surface of solid media only under anaerobic conditions upon primary isolation from an infectious process, obligate anaerobic hemolytic streptococci are rarely encountered. We have isolated only three strains that would grow anaerobically only after 10 transplants on solid media. Two were from lung abscesses, and one from a wound infection following an appendectomy; one strain belonged to Lancefield Group A, the other two to Group F. Morphologically and culturally they did not differ from the faculative aerobic strains of hemolytic streptococci except in their inability to form surface colonies on solid media under aerobic conditions.

(2) *Nonhemolytic Streptococci:* The first isolation of an anerobic nonhemolytic streptococcus is generally accredited to Veillon,[159] who in 1893 described a strictly anaerobic coccus that grew in short chains and produced gas and fetid odor in cultures. He called this organism *Micrococcus foetidus,* and it was referred to as such until Prévot[160] demonstrated in 1924 that it was a streptococcus. This and other species of anaerobic nonhemolytic streptococci have been recovered from diverse infectious processes and from normal body cavities. Sandusky and other members of the author's laboratory staff presented a table of the normal habitat, sources of infection, and diseases from which anaerobic nonhemolytic streptococci have been isolated, in a review of their role in surgical infections.[161]

There is no satisfactory answer to the question whether or not anaerobic nonhemolytic streptococci are true pathogens. There is no doubt that in certain instances the pathogenic role is assumed only when the tissues of the host have been altered either by trauma, necrosis, diminished blood supply, or chemical changes. However, there is considerable evidence pointing to the fact that they are definite pathogens to man, either alone or in symbiosis with other bacteria. Their role in puerperal infections—when, in some instances, it is possible to recover the organisms in pure culture from thrombosed pelvic veins, the blood stream, and distant metastatic abscesses—has been recognized by Schottmuller,[162] Harris and Brown,[163] Colebrook,[164] and others. MacDonald, Henthorne, and Thompson[165] believe that the frequency in which they were found in pure culture makes it necessary to conclude that they play a significant role in all cases in which they are present. In the

TABLE I[161]

THE NORMAL HABITAT, SOURCES OF INFECTION, AND DISEASES FROM WHICH THE ANAEROBIC NONHEMOLYTIC STREPTOCOCCI HAVE BEEN RECOVERED

Oral Cavity		
Normal flora	Retropharyngeal abscesses	Cerebral abscesses
Normal sinuses	Deep cervical abscesses	Aspiration pneumonia
Gingivitis	Ludwig's angina	Pulmonary gangrene
Ulcerative stomatitis	Sinusitis	Lung abscesses
Dental caries	Mastoiditis	Empyema thoracis
Tooth granulomas	Otitis media	Septicemia
Alveolar abscesses	Leptomeningitis	Tooth wounds
Tonsillitis		Infected carcinoma
Gastrointestinal Tract		
Normal intestinal flora	Liver abscesses	Putrid enteritis
Acute and chronic cholecystitis	Acute appendicitis	Anorectal infections
	Appendiceal abscesses	Wound infections
	Acute peritonitis	
Female Genitourinary Tract		
Normal flora	Bartholinitis	Metastatic abscesses
Foul lochia	Salpingitis	Thrombophlebitis
Endometritis	Tubo-ovarian abscesses	Septic abortion
Parametritis	Peritonitis	Bacterial endocarditis
Cervicitis	Infected carcinoma	Pulmonary gangrene
	Septicemia	
Miscellaneous		
Accidental wounds	Postoperative wound infections	Pyonephrosis
War wounds	Intertrigo	Perinephritic abscesses
Gas gangrene	Periurethral suppurations	Osteomyelitis

group of surgical infections reported by Sandusky et al, these streptococci were recovered in pure culture in 29 out of the 158 cases studied.[161]

The anaerobic nonhemolytic streptococci play a part in the bacterial flora of acute, suppurative, gangrenous, and perforative appendicitis with peritonitis and abscess. Together with the fusospirochetal group of organisms, they have an important synergistic role in the production of lung abscesses and gangrene, and infections following human bites or tooth extractions. In certain cases of myositis in which crepitation can be felt and heard in the tissues, but where no clostridia are found, the production of gas may be attributed to a gas-forming anaerobic nonhemolytic streptococcus in the mixed bacterial flora. Eight such cases have come under our observation and similar ones have been reported by MacLennan[166] and others

TABLE II [161]

29 CASES FROM WHICH THE ANAEROBIC NONHEMOLYTIC STREPTOCOCCUS WAS ISOLATED IN PURE CULTURE

	Cases
Perirectal abscess	9
Infected pilonidal cyst	4
Infected sebaceous cyst	3
Empyema thoracis	2
Cholecystitis	2
Abscess of thumb	1
Infected branchial cyst	1
Brain abscess	1
Appendiceal abscess	1
Intraperitoneal abscess	1
Liver abscess	1
Pericholecystic abscess	1
Lung abscess	1
Postoperative wound infection	1

(a) *Morphology.* Morphologically the majority of anaerobic nonhemolytic streptococci are not strikingly different from the aerobic varieties. However, there is considerable difference in the size of the individual cocci of different strains and in the length of chains formed. One species, *Streptococcus foetidus,* divides to form parallel chains.[160]

(b) *Cultural Characteristics.* There is a considerable variation in the cultural characteristics of these streptococci. Some so-called species produce gas and a fetid odor in liquid media, others do not. They may grow diffusely or with a granular or viscous sediment. The coagulation of milk by them is also variable. Colonies on blood-agar plates are small, round, and translucent, with the exception of one species (parvula), which elaborates a black pigment.

(c) *Classification.* There is at present no adequate system of classification. Prévot [160] in 1925 was the first to attempt a classification on the basis of morphological and cultural characteristics. He divided the streptococci into two major groups, those that produced gas and fetid odor in liquid media, and those that did not. Each of these groups was further divided into three species; on the basis of production of general turbidity in broth, size of individual cocci, coagulation of milk, and so on. Although these would seem to be valid species, it has been the common experience of all workers to find that many strains are isolated that do not fall into any one of these divisions. Sandusky et al.[161] reported that out of 37 strains in which classification was attempted, 23 had biochemical reactions that did not agree with those outlined by Prévot. Colebrook and Hare [167] attempted a classification on the basis of morphology and colony appearance on horse-blood agar and

was it present in pure culture, and its role in the infection is diffic assay. These staphylococci are normal inhabitants of the mouth intestinal canal, and the genitourinary tract.

(1) *Morphology:* The anaerobic staphylococci grow in irregular m similar to those of the aerobic staphylococci. Different species considerable variation in the size of the cocci, and Prévot has such differences as one of the bases for classification.[169]

(2) *Cultural Characteristics:* Different species vary in the producti general turbidity in broth, in the formation of gas and fetid od the fermentation of glucose, and in indol production. No enrich of the usual laboratory media is required. We have found it advi to continue the anaerobic incubation for forty-eight hours, as in experience, these organisms grow slowly. The strains we have iso have all been strict anaerobes. Colonies on the surface of blood plates are small and round and may be either transparent or some opaque.

(3) *Classification:* Prévot[169] lists three species differentiated by: (1 production of gas (*Staphylococcus anaerobius*); (2) production of and fetid odor (*Staphylococcus aerogenes*). *Staphylococcus asacc lyticus* is further differentiated from *Staphylococcus aerogenes* by large size of the individual cocci, 1 to 1.2 μ in diameter.

(4) *Animal Pathogenicity:* There are a few reports of individual st producing abscesses in rabbits, guinea pigs, and mice, but the evid of pathogenicity for laboratory animals is far from conclusive.

2. *Gram-negative Cocci*

A. NEISSERIA

Prévot[169] lists three species of anaerobic Gram-negative cocci grov in pairs in 'coffee-bean' morphology. These organisms are not comm found in surgical infections.

B. VEILLONELLA

The pathogenic significance of Veillonella is still to be determir but they are of frequent enough occurrence in surgical infections warrant their inclusion here. They are natural inhabitants of the mo and alimentary canal of man and of animals. They are small cocci liquid media, which grow in irregular masses. One species (*V. parv* produces gas with a slightly disagreeable odor. On blood-agar plates tiny colonies are sometimes surrounded by a zone of weak hemoly It has been isolated from suppurative lesions of the mouth, lungs, a

appendix. Although normally a saprophyte, in some cases it invades the tissues and causes suppuration. It has been recovered in pure culture from such pyogenic infections, but more often it is found associated with other organisms. Another species (*V gazogenes*) forms in liquid media abundant but odorless gass. The minute colonies on anaerobic blood-agar plates are nonhemolytic as a rule although occasionally there may be a green discoloration of the agar. Hall[173] reports the isolation of twenty-four strains from human saliva. It has been cultured from pyorrhea, tonsillar infections, lung gangrene, and appendicitis Prévot[169] reports that two of his strains were pathogenic for rabbits.

B. ANAEROBIC BACILLI

1. *Gram-positive Sporulating Bacilli (Clostridia)*

The great majority of the anaerobic Gram-positive sporulating bacilli are saprophytes and may live and even multiply in wounds without increasing the virulence of the infection. Those that are pathogenic, however, are of the utmost importance and will be considered here.

A. HISTOTOXIC OR GAS-GANGRENE GROUP

In this group are included the true pathogens, those that can produce 'gas gangrene' when present in the wound in pure culture, and certain other species that are nontoxic in pure culture but that greatly increase the virulence of the infection if they are present in association with the true pathogens.

To the first group belong the etiological agents of 'Clostridial gas gangrene–*C. welchii, C. novyi, C. septicum, C. sordellii,* and *C. histolyticum.* The hystolyticus bacillus is a microaerophilic organism, but because of the character of the lesion it produces, it seemed best to discuss it with the 'gas-gangrene' organisms. To the second group–non- or opportunist pathogens–belong such organisms as *C. sporogenes* and *C. bifermentans.*

(1) *Clostridium welchii:* This organism was first adequately described by Welch and Nuttall[174] in 1892 and is generally regarded as the common cause of gas gangrene, as it occurred in civil practice before World War I. During the war, however, it was frequently found to exist in wounds without causing any infection; and when infection was present, it was almost invariably accompanied by other organisms, in some cases harmless in themselves, but with very evident power to enhance the virulence of the Welch bacillus or be themselves enhanced by it. In a large series of cases of gas gangrene studied by Weinberg and Séguin[175] during World War I, *C. welchii* was found in from 70 to 80 per cent of them. MacLennan[176] in reporting a series of 146 gas-

gangrene cases in the Middle East during World War II, found *C. welchii* the only pathogenic clostridium in 50 cases. In an additional 33 cases, it was associated with other clostridia, either pathogens or opportunist pathogens. Meleney reports its occurrence in the débrided tissue removed from civilian accident cases as follows: from 926 soft-part wounds, it was recovered 138 times; from 674 compound fractures, 147 times; from 591 burn cases, 175 times. In only a small fraction of these cases did it take part in subsequent infection.[177] The Welch bacillus is also found in diabetic and arteriosclerotic gangrene of the extremities, and a gas gangrene may develop after amputation. It is also found in infected lesions associated with the intestinal tract—appendiceal and other intra-abdominal abscesses, in diffuse peritonitis—and in postoperative wound infections following surgery on this tract. It has been recovered from thoracic fluid, where it seldom forms gas, and from bile when there is obstruction of the common duct. It is also occasionally present in cases of acute pancreatitis.

Clostridium welchii has been found almost universally in the intestinal tract of man and of animals. Because of its ability to form spores it may live in the soil for a long time, and is distributed widely by dust and dirt.

(a) *Morphology. Clostridium welchii* is a large, thick, Gram-positive bacillus with blunt or slightly rounded ends; 4 to 8 μ by 0.8 to 1.0 μ. It usually occurs singly or in pairs. It is surrounded by a fairly thick capsule. It is nonmotile. Spores are large, ovoid, and occupy a central to excentric position in the rod. They are rarely seen in animal exudates and in culture medium are formed only when the pH of the medium is above 6.6 (see Fig. 12).

(b) *Cultural Characteristics. Clostridium welchii* is less sensitive to free oxygen than most of the anaerobic clostridia.It grows well in cooked-meat medium if the medium is boiled and then cooled rapidly just prior to inoculation. A considerable amount of gas is evolved, and the meat particles may be blown to the top of the tube. Cooked-meat medium and brain medium are slowly blackened.[178] In milk, a characteristic 'stormy fermentation' takes place, usually within 8 to 24 hours of incubation. This characteristic action on milk was noted by Welsh and Nuttall[174] in their first description of the bacillus. The clot is ruptured by the violent gas formation, and the firmly retracted clot separates from the whey. An occasional strain may fail to show this reaction or it may be delayed for 48 hours. There is, at times, some variation in the sugar fermentations and in the reduction of nitrates to nitrites. *Clostridium welchii* is feebly proteolytic.

An important differential characteristic of *C. welchii* strains is the production of acrolein in 2 per cent glycerol broth. The acrolein kills



FIG. 12 Guinea pig killed in 16 to 20 hours by the injection of 0 5 cc of a culture of *C. welchii* into the left thigh. Note the exposed vessels of the groin The muscle was disintegrated and a large sac of gas filled the groin (1¼ life size.) *Insert. C. welchii* from blood-agar plate, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 13. Welch colonies *C. welchii*, both hemolytic and nonhemolytic types, on sheep's blood agar. The hemolytic types have an inner clear zone of complete hemolysis (due to theta toxin) and a wide outer hazy zone of partial hemolysis (due to alpha toxin). The nonhemolytic types have only the outer zone

not only the Welch bacillus but any or all organisms associated with it in the culture. Humphreys[179] states that he has never encountered a strain that failed to give this reaction, or any other anaerobic bacterium that produced acrolein.

Because spores are rarely formed in the usual laboratory media, young cultures are less resistant to heat than those of the other 'gas-gangrene' organisms. The spores themselves, however, are also less resistant, not surviving at 100° C. for more than five minutes.[180]

On the surface of blood-agar plates *C. welchii* forms colonies 1 to 3 mm. in diameter after twenty-four hours' incubation. They are convex, round with a smooth edge, and of a creamy, translucent color. In the hemolytic type the colony is surrounded by an inner zone of clear hemolysis and an outer zone that is slightly darker than the blood-agar medium. This 'double-zone' phenomenon gives a characteristic 'target' appearance, with the colony as the bull's eye, which is produced by no other clostridium as far as we know (Fig. 13). In the nonhemolytic types, the inner, clear zone is missing and the colony is surrounded by a broad, darker zone only. This differentiation is best seen on sheep- or human-blood-agar plates. The 'double-zone' phenomenon does not occur with rabbit's blood.

(c) *Toxin Production*. *Clostridium welchii* elaborates in vitro three antigenically distinct toxins—alpha toxin,[181] theta hemolysin,[182-3] and hyaluronidase or spreading factor[184]—and a nonantigenic fibrinolysin.[185] The alpha toxin is hemolytic and destroys leucocytes. It is believed

to be identical with lecithinase.[186-7] It is produced by all strains *in vitro*, but the amount produced varies considerably.[188-9] The amount of alpha toxin produced *in vitro* has been correlated with the virulence of the strain for guinea pigs. Strains that produced only small amounts of alpha toxin did not cause fatal infections, while the majority of those with better alpha-toxin-producing powers caused fatal infections readily.[189]

Theta hemolysin is not elaborated by all strains *in vitro*. Ten of the 30 strains examined by Evans[189] failed to produce it, although 4 of the 10 caused fatal infections in guinea pigs.

Hyaluronidase is not produced by all strains. McClean et al.[190] report that the large majority of strains isolated from gas gangrene in man produced hyaluronidase although they failed to detect this enzyme in cultures of certain strains toxigenic for man and for guinea pigs. They believe that hyaluronidase production is connected with the invasiveness of the strain. Evans[189] found that 4 out of 9 strains isolated from gas-gangrene infections failed to produce it in detectable amounts. All 9 strains caused fatal infections in guinea pigs.

Evans[189, 191-2] also found that the alpha antitoxin was the significant factor in the protection of guinea pigs against experimentally produced gas gangrene, as antisera with only traces of alpha antitoxin did not protect the animals even though considerable amounts of theta anti-hemolysin and antihyaluronidase were present. Antisera that contained potent alpha antitoxin gave good protection in the absence of anti-hemolysin and antihyaluronidase.

(d) *Animal Pathogenicity. Clostridium welchii* is extremely pathogenic for sheep, guinea pigs, mice, and pigeons. Rabbits are somewhat more resistant. When 0.1 to 0.2 cc. of a 24-hour culture is injected into the thigh muscles of a guinea pig, the animal usually dies within 12 to 24 hours, with an intense edema of the muscles and the production of gas, which forms a large cavity beneath the skin of the groin, extending on to the abdomen. The muscles have a typical 'cooked-meat' appearance with a dark-red serous exudate. Some strains kill the animal by toxin produced in the local lesion, while others rapidly invade the blood. These strains have been called, respectively, toxic and invasive.

(2) *Clostridium novyi:* Weinberg and Séguin[175] recovered this bacillus from 34 per cent of the wounds they examined during World War I. It is generally accepted as being the same organism as that described by Novy[183] in 1894 under the name *Bacillus oedematis maligni II*. It has not been found in infectious processes in man other than in wounds. MacLennan[176] isolated it in 55 out of 146 cases of gas gangrene in the Middle East campaign of World War II. It was the second most com-

mon pathogenic clostridium recovered, *C. welchii* being the most common. In these war wounds it is undoubtedly more virulent than *C. welchii*, and in many cases both organisms are found.

It is rarely present in the intestinal canal of man but is found in heavily fertilized soil. Zeissler and Rassfeld [194] also found it in sausages, preserved meats, and factory refuse.

(a) *Morphology. Clostridium novyi* is a large Gram-positive bacillus, 0.8-1 μ by 3-10 μ, with rounded ends. It occurs singly, in pairs or in chains. The spores are large, oval, subterminal and swell the rod. They are formed freely in all media, and are present even in young cultures. The organisms possess many flagella and are actively motile in young cultures. The bacilli in young cultures are Gram-positive, but in older cultures may be Gram-negative (see Fig. 14).

(b) *Cultural Characteristics. Clostridium novyi* requires strict anaerobiosis. It grows readily in cooked-meat medium or in mashed-brain broth in the anaerobic jar or under a 'vaspar' seal, but sparsely or not at all in the cooked-meat medium under aerobic conditions. Gas with a rancid odor is produced, but the meat is not digested. After twenty-four hours, the growth tends to settle as a heavy cloud, often leaving the supernatant fluid clear. There is inactive gaseous fermentation in milk, but clotting may not occur unless incubation is continued for twelve days or longer. Different strains may vary somewhat in their fermentation reactions but are alike in their acid production in glycerol. Gelatin is liquefied, but coagulated serum is not affected.

The spores as a rule are more heat-resistant than other species of anaerobic clostridia, but are usually killed by moist heat at 100° C. in twenty to thirty minutes. Young spores are less resistant. Differential heating of mixed cultures is of value in the isolation of this bacillus, since it often survives 90° C. for fifteen minutes.

On blood-agar plates, the colony formation is very variable. At times, especially on moist plates, the colonies may have a spreading character. The most common form is flat, grayish, with deeply undulated margins. There is usually a narrow zone of partial hemolysis or methemoglobin formation.

(c) *Toxin Production. Clostridium novyi* produces a potent antigenic exotoxin containing a hemolysin. It kills mice in doses as small as 0.001 cc. One unit of the standardized antitoxin will protect mice against 5000 M.L.D. The polyvalent gas-gangrene antiserum prepared according to the International Standards established by Bengston,[195] Stewart and Bengston,[196] and Bengston and Ipsen [197] has been shown by Hall [198] to contain sufficient antitoxin in the 'therapeutic dose' to neutralize 7,500,000 mouse M.L.D. For the efficacy of this serum in combating human infections, the reader is referred to Chapter XXIV.



FIG 14. Guinea pig killed in 16 to 24 hours by the injection of 0 5 cc of a culture of *C novyi* (*B. edematiens*) into the left thigh Note the widespread edema that involves the *opposite groin and all of the abdominal wall It is colorless and jelly-like.* An incision has been made on the abdominal wall through the edema to show its depth (6 to 7 mm). An areolar vesicle is seen at the side (Life size) *Insert C. novyi* culture in meat-dextrose broth, × 1500.

McClean et al.[190] found that only 7 out of the 15 strains of *C. novyi* tested produced hyaluronidase, and this production could not be correlated with the ability of the strain to produce gas gangrene. They also concluded that the lecithinase produced by this organism was of too low a potency to be of diagnostic significance in the tissue fluids.

It has been demonstrated by Reed, Orr, and Brown[185] that an active fibrinolysin is formed by *C. novyi.* All of the strains tested produced this thermostable lysin. It is not related to the proteolytic enzymes of the culture and is nonantigenic.

(d) *Animal Pathogenicity.*[108] *Clostridium novyi* is extremely pathogenic for all laboratory animals. When 0.1 to 0.25 cc. of a twenty-four-hour culture is injected into the thigh of a guinea pig, it produces a slightly hemorrhagic gangrene of the muscle with comparatively little gas. However, there is a jelly-like edema of the subcutaneous tissues. This edema spreads upwards, frequently extending over the abdomen and chest (see Fig. 14). Smears made of this edematous tissue reveal very few organisms. Weinberg and Séguin believe this edema to be a reaction to a toxin.[175] The animal may show no symptoms other than a local swelling for ten to twenty-four hours and then die suddenly from what appears to be a general intoxication. At autopsy the organisms may be recovered from the local lesion, the peritoneum, and the heart's blood. The edematous fluid is often sterile. Strains differ in the production of this jellylike edema, although all produce it to some extent. As with the Welch bacillus, some strains seem to be more toxigenic, others more invasive. If the necropsy is performed on a guinea pig immediately after death, and if the toxic manifestations have been severe, the blood culture may be negative.

(3) *Clostridium Septicum (Vibrion Septique): Vibrion septique* was observed and described in a limited way by Pasteur in 1877,[199] and enjoys the distinction of being the first of the virulent anaerobic bacteria to be recognized. Weinberg and Séguin[175] found it in 12 per cent of the wounds examined by them, but it was present in pure culture in only one. MacLennan[176] recovered it from 28 out of 146 cases of gas gangrene occurring during the Middle East campaign, it was the only pathogenic clostridium present in 7 cases. It is not common in infections arising from the intestinal tract in man, but we have recovered it in pure culture from postmortem cultures of two cases of rapidly fatal peritonitis following operations on the large bowel. Humphreys[200] found it in two cases in which unsterile catgut was the source of infection. It is found in manured soil, but is a much less common inhabitant than *C. welchii* or *C. novyi.*

(a) *Morphology. Clostridium septicum* is a large Gram-positive bacillus, 2-6 μ by 0.4 to 0.6 μ. In cultures it is usually single, in pairs, or in short chains, but Weinberg and Séguin have emphasized that the characteristic form in the peritoneal exudate of guinea pigs and mice dying of the infection is long, jointed filaments. These threads can be found regularly on the surface of the liver.[175] Spores are readily formed, are oval and subterminal, and bulge the rod. Free spores are often seen. It is actively motile in young cultures and in tissue exudates. No capsule has been demonstrated. Although Gram-positive in young cultures, in older cultures it may be Gram-negative (see Fig. 15).

(b) *Cultural characteristics. Clostridium septicum* requires the same strict anaerobiosis as does *C. novyi.* It grows well in cooked-meat medium and in brain medium, but the media are neither blackened nor digested. Gas production is greater than with *C. novyi,* but less than with *C. welchii.* Milk is acidified but clot production is slow, with no subsequent digestion. It is saccharolytic and only feebly proteolytic. On blood-agar plates it frequently spreads rapidly from the original site of implantation and may cover the whole plate. This spreading proclivity makes it difficult to recover other organisms growing with it, although from the margin of the spread this organism may be easily obtained in pure culture. Clostridia more resistant to heat—*C. novyi* for example—may be separated from it by selective heating. The majority of strains are hemolytic.

(c) *Toxin Production. Clostridium septicum* produces an antigenic soluble exotoxin, containing both a hemolytic and a lethal factor.[201] It still remains to be determined whether these two factors are identical. McClean et al.[190] found that all strains they examined contained hyaluronidase though in much smaller quantities than in hyaluronidas-positive *C. welchii* strains. In *in vivo* experiments in guinea pigs, as the infection progressed, there was a rapid increase in the hyaluronidase content of the edema fluid in comparison with that in the muscle.

Pasternack and Bengston[202] have reported that *C. septicum* toxin when injected intravenously into rabbits kills them almost instantaneously. They believe that this toxin has powerful cardiotoxic properties, if not an especial affinity for the heart. Neutralization of the toxin with an antitoxin protected the animals.

Laboratory animals can also be protected against gas gangrene by the antitoxin.

Of the strains tested by Reed, Orr, and Brown[185] for the presence of fibrinolysin, 83 per cent lysed coagulated plasma from man, guinea pigs, rabbits, and sheep. They also demonstrated that muscle from a *C. septicum* infection contained an active fibrinolysin.



FIG. 15. Guinea pig killed in 16 to 20 hours by the injection of 0 5 cc. of a culture of *C septicum* (*Vibrion septique*) into the left thigh Note the extensive swelling and edema of the tissues of the groin of that side. It had a bright red hemorrhagic color at necropsy (1½ life size.) *Insert:* Long thread and other forms in peritoneal exudate on the surface of the liver, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

(d) *Animal Pathogenicity.*[104] *Clostridium septicum* is extremely virulent for guinea pigs, mice, and pigeons but rabbits are more resistant. When injected into the thigh of a guinea pig, it produces a characteristic rosy red color of the muscle, with a bloody exudate and edema. There is very little gas formation. At autopsy, the organisms are found in great numbers in the lesion, the peritoneal cavity, and blood (see Fig. 15).

(4) *Clostridium sordellii* (*Clostridium œdematoides*): This organism was isolated and reported as a new species in 1927 by Meleney, Humphreys, and Carp.[203-4] It had been described briefly in 1922 by Sordelli,[205] who sent his strain to Hall[206] for more careful study. Hall's observations, which were made after Meleney's report, suggested to him that the two organisms belonged to the same species, and this was later demonstrated by reciprocal toxin-antitoxin tests.[207-8] To Sordelli, therefore, goes the priority of discovery. The first strain to be studied by Meleney was cultured from the lesion in a fatal operative-wound infection. Four other patients who were operated on during the same period died from wound infection, all simulating gas gangrene. The catgut was suspected and, when examined for anaerobic organisms, yielded 4 or 5 different types of spore-forming anaerobic organisms as well as one aerobic spore-former. Two strains of *C. sordellii* were found in 2 out of 4 tubes of catgut examined. This clostridium is not a common organism, but Hall has recovered it on two different occasions.[209-10] We have recently isolated it from the point of origin of fatal cellulitis following a hypodermic injection.[211] *Clostridium welchii* was also present in the lesion. MacLennan[176] refers to a toxic *bifermentans*, and it is probable that this is *C. sordellii* as the morphology and cultural characteristics of the organisms are similar.[212] Spray[213] differentiates them on the basis of pathogenicity only.

(a) *Morphology.*[108] *Clostridium sordellii* is a Gram-positive rod, 0.5 to 1.5 μ by 1.5 to 7 μ. They are usually single but may form chains of four to five units. The spores are oval, excentric to subterminal, and bulge the rod (see Fig 16a). Spores are readily formed and in 48-hour cultures on blood-agar plates, the colony may consist almost entirely of free spores. It is motile in young cultures.

(b) *Cultural Characteristics. Clostridium sordellii* grows readily under anaerobic conditions. In cooked-meat medium there is abundant growth with digestion of the meat particles. Both cooked-meat medium and brain-mash broth are blackened, especially if iron is added to the medium. In milk containing iron wire, the milk is blackened and digested. *Clostridium sordellii* has neither an active saccharolytic nor proteolytic action On blood-agar plates after twenty-four hours' incu-

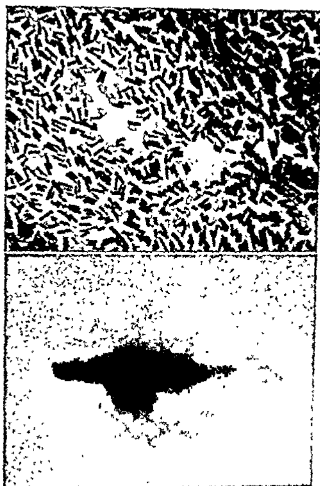


FIG. 16. (a) *C. sordellii* in a smear from a blood-agar plate, 20 hours' growth. Large Gram-positive spore-forming bacillus, with spores paracentral when in the bacilli, but most of them free, × 1000.

(b) Colony of *Clostridium sordellii* (*C. oedematoides*) on a blood-agar plate, 20 hours' growth. The colony is usually stellate with the long diameter in the direction of the streak of the inoculating needle, × 35

bation, the colony is grayish and translucent and has a characteristic stellate shape (Fig. 16b). After forty-eight hours or more, when the colony consists of a preponderance of free spores, it is opaque and a pale buff in color. There is no zone of hemolysis around the colony, but sometimes a greenish tinge after the plate has stood at room temperature for a few hours after anaerobic incubation.

(c) *Toxin Production. Clostridium sordellii* produces a soluble antigenic exotoxin.[203, 206] Walbum and Reyman[214] found that the maximum titer was reached after eight to twelve days' incubation, and then declined. Reed, Orr, and Brown[185] demonstrated the presence of an active fibrinolysin formed by this clostridium. Its properties were the same as the fibrinolysins produced by the other 'gas-gangrene' clostridia.

(d) *Animal Pathogenicity.*[108] Small doses injected subcutaneously or intraperitoneally cause rapidly fatal infections in mice, rats, guinea pigs, rabbits, cats, dogs, pigeons, and chickens (see Fig. 17). There is an extensive hemorrhagic edema of the subcutaneous tissues, resembling somewhat the lesions produced by *C. novyi* and *C. septicum*. As in

C. novyi infections, the animals may show no marked symptoms e: for a swelling at the site of injection until just before death, whi preceded by a complete collapse. Hall [215] has found that the orga when very toxic does not invade tissues extensively, and in ord recover the bacillus, cultures must be taken at the site of the ori infection.

(5) *Clostridium sporogenes: Clostridium sporogenes* was first desc by Metchnikoff in 1908.[216] Although *C. sporogenes* is not of pathogenic for man, it undoubtedly enhances the pathogenicity of anaerobic clostridia in gas gangrene, probably because of its a putrefactive properties.[217] It has been shown that while the additic *C. sporogenes* to cultures of the nonproteolytic clostridia, such a *welchii* and *C. novyi*, reduces the minimum lethal dose of such cul for animals, the addition of a filtrate of *C. sporogenes* to toxic filtrat *C. welchii* and *C. novyi* has no such action.[218] It is a common cont nant of accidental wounds, owing to the fact that it is widely distrib in the soil and a normal inhabitant of the intestinal tract of man of animals.

(a) *Morphology. Clostridium sporogenes* is a large strongly G positive motile bacillus. The spores are oval, subterminal, and disti swell the rod. They are freely produced in both liquid and on media.

(b) *Cultural Characteristics. Clostridium sporogenes* grows readi cooked-meat medium and in brain medium, digesting and blacke the meat.[176] It also blackens iron milk rapidly with a digestion of



FIG. 17. The lesion of the toxin of *Clostridium sordellii* (*C. œdematoides*) in mice. The mouse on the right died overnight with a large dose of toxin. That on the left died in 3 days with a small dose of toxin. The edema is usually greatest when death occurs slowly.

clot. The most important cultural characteristic from the point of view of biologic activity is the active proteolytic action on both gelatin and coagulated blood serum.

On blood-agar plates, the colonies are flat and rhizoid and are surrounded by a narrow zone of hemolysis. After 48-hour incubation, the colonies are whitish yellow and resemble a piece of raveled wool.

Some of the strains are very resistant to heat, and spores may withstand boiling for an hour or more.[219] These organisms have frequently been the cause of error in the description of the cultural characteristics of other anaerobic bacilli because of the heat resistance of the spores and the slowness of germination of some of the spores. They may also grow in colonial symbiosis with other sporulating bacilli for many generations.

(c) *Toxin Production.* There is no proof that *C. sporogenes* forms a toxin capable of producing antitoxin when injected into animals. Reed, Orr, and Brown[185] found that 58 per cent of the strains tested produced a fibrinolysin active against coagulated plasma from man, guinea pigs, sheep, and rabbits. This fibrinolysin, however, is nonantigenic, in which respect it differs from that formed by hemolytic streptococci. Filtrates of *C. sporogenes* cultures also contain an actively proteolytic enzyme, which liquefies gelatin and digests heated muscle but not the raw tissue.

(d) *Animal Pathogenicity.*[108] For guinea pigs and other laboratory animals *C. sporogenes* is essentially nonpathogenic, although some strains produce on subcutaneous injection a slight swelling, which usually disappears within a few days When a mixture of *C. welchii* and *C. sporogenes* is injected, both organisms may be recovered from the blood on postmortem examination. This is apparently another demonstration of the symbiotic action of these two organisms. In man, except for the 'opportunist' action in mixed infections, *C. sporogenes* is not pathogenic although certain forms of diarrhea have been attributed to it.[220]

(e) *Related Species.* Certain other putrefactive organisms, such as *C. bifermentans* and *C. capitovalis* are closely allied to *C. sporogenes* in their cultural characteristics. These are essentially nonpathogenic.

B. NEUROTOXIC SPECIES

(1) *Clostridium tetani:*[108] Although the infection produced by this organism is essentially an intoxication of the nervous system and therefore not amenable to surgery, the tetanus bacillus is of the greatest interest to surgeons because of its frequent occurrence in wounds grossly contaminated with dirt. In every war before 1914, tetanus infections produced a high mortality rate among the soldiers, but in World

ar I this scourge was controlled to some degree by the extensive tetanus antitoxin.[221-2] The development of tetanus in the prim ound was minimized by the injection of the antitoxin as soon as edical attendant reached the wounded. However, tetanus develop t infrequently following secondary operations on wounds, when nporary immunity conferred by the antitoxin had been lost and wh e organisms still present in the wound were given a favorable opp nity for growth. In World War II, tetanus was practically eliminat cause of the prophylactic active immunization of the military p nnel of the allied armies with tetanus toxoid before they left ho ses and a 'boosting dose' at the time of injury. Of the few cases t curred, the great majority were in unimmunized subjects. Tetanus id to have been very prevalent among the German soldiers, howev ho did not have active immunization.[223]

In civilian practice, tetanus infections were formerly common, p cularly in puncture wounds produced by dirty objects on the fa roadside, in puerperal infections, and in infections of the navel e newborn. At the present time, tetanus is rarely seen, owing in p the replacement of the horse by the automobile, but in greater p the widespread use of tetanus antitoxin as a routine procedure in eatment of accidental contaminated wounds.

The organisms are rather infrequently found in the intestinal tract an in Europe and America. In England, Tulloch[224] found that o eries of soldiers home on leave during World War I, 83 per c arried tetanus bacillus in the intestine, whereas of civilians who h ot been at the front, 16 per cent carried the organism. It has b egularly found that individuals closely associated with animals, fa orkers, for instance, have *C. tetani* in the intestinal tract. Park a Villiams[225] have reported that 15 per cent of horses and calves in icinity of New York City carry tetanus bacilli in the intestines, a Ioble's[226] series gives a percentage of 18. Tenbroeck and Bauer[227] ha emonstrated that among Chinese civilians, infestation runs as high 3 per cent. The organisms unquestionably multiply in the intesti ract and may contaminate any uncovered wounds around the anus. China, Meleney observed a number of cases of tetanus followi acral bed sores and one case following a gun shot wound of bdomen, cases to which Tenbroeck and Bauer refer.

(a) *Morphology.* The tetanus organism is a thin Gram-positive bacill arying considerably in length It may exist as long filaments t tretch across the microscopic field and later undergo segmentati Iotility is due to a large number of peritrichious flagella. It forms haracteristic spore, which is round and terminal and gives the organi

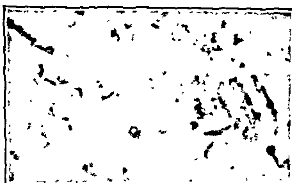


FIG. 18. *C. tetani* from a n dextrose broth culture, sho the round terminal spores, × 1 (From Nelson's *Loose-Leaf gery*. Courtesy of Thomas Ne and Sons)

a 'drumstick' appearance. The spores appear early, and in old cult the organisms are almost entirely in the spore form (see Fig. 18).

(b) *Cultural Characteristics*. *C. tetani* requires strict anaerobiosis. grows readily in cooked-meat medium. As a rule, no change is produ in milk, although prolonged incubation may occasionally cause coagu tion without acidification. It ferments no sugars, but gelatin is slo liquefied. On blood-agar plates, it produces colonies that spread a form a film over the surface of the media.

The spores are resistant to heat and may resist boiling for fifteen ninety minutes or more.[228]

(c) *Toxin Production*. The tetanus bacillus elaborates two filtral antigenic toxins, a hemolysin—tetanolysin, and a neurotoxin—tetar spasmin. The hemolysin seems to be of little pathogenic importance, b the neurotoxin is one of the most potent poisons known. It is respo sible for the characteristic muscle spasms in tetanus resulting from intoxication of the cells of the nervous system. The muscles around t site of entrance of the bacillus into the body are usually the first to involved, but the frequency with which the muscles of the mouth a jaw are affected has given rise to the popular name 'lockjaw.' As t toxin is disseminated through the nervous system, general convulsio occur, followed by death due to asphyxia.

In broth cultures of the bacillus that have been incubated anaerobicall at 37° C., toxin is formed within twenty-four hours, and increases i amount up to 10 to 14 days. Mueller and Miller[229-30] investigated tox formation in synthetic media and found that the iron content was a important factor. The most potent toxins were obtained when as muc iron as possible was removed from the medium. The toxin is withou effect if given orally, but if it is injected systemically, unbelievably sma amounts produce the characteristic symptom-complex in susceptible ani mals. Hall[231-2] has reported the production of toxic broth filtrates with a minimum lethal dose for guinea pigs of less than 0.0001 cc. Partially purified aluminum-sulphate precipitates of toxic filtrates have been re-

ported to kill mice in doses of 0.000,000,05 gm. and guinea pigs in doses of 0.000018 gm.[233-4]

The antitoxin that will neutralize this poison was one of the first studied by Behring and Kitasato.[235] The effectiveness of tetanus antitoxin as a prophylactic was amply demonstrated during World War I. Before the general use of the antitoxin, there was a high incidence of tetanus among the wounded soldiers, but there was a rapid fall in the number of cases after its adoption for every wounded soldier at the earliest possible time after injury. Tetanus antitoxin is of considerably less value in the treatment of the disease after the symptoms have appeared, but should be given in large quantities intravenously, intramuscularly, and intraspinally when earliest signs of the disease manifest themselves. The antitoxin cannot neutralize toxin already fixed in the central nervous tissue, but can combine with any free toxin in the blood stream or newly manufactured toxin at the site of bacterial activity.

(d) *Animal Pathogenicity.* A great deal of investigation has been carried on with regard to this interesting organism. When spores are injected, they often fail to kill the animal unless there is at the same time some injury to the tissue in which they are injected. When the vegetative forms are inoculated, they are extremely virulent for mice, guinea pigs, rabbits, and a number of other animals, but rats and birds are relatively insusceptible. Certain strains have a relatively long incubation time before the typical symptoms of tetanus develop. It has been found that this incubation time has two phases: first, the local production of toxin; second, the attachment of the toxin to the susceptible cells of the muscles or the central nervous system. Until recent years, the theory has been generally accepted that the toxin produced locally in the region of the wound reaches the central nervous system only by passing up the motor neurons to the anterior horn cells of the cord and thence spreading both up and down within the cord and backward to involve the sensory nerve cells. It was thought that whatever toxin was absorbed by the blood and lymph was distributed by the arterial circulation to the various muscles of the body and then, in turn, absorbed by the axis cylinders of their motor cells. The absorption was said to be accomplished either by 'protoplasmic streaming' within the axis cylinder, by way of the perineural lymphatics, or up through the interfibrillar channels. The symtoms of descending or general tetanus beginning in the face and jaw muscles was explained by the absorption of the toxin by the motor nerves in these muscles with contraction occurring as soon as the toxin reached the motor nerve cells in the cord.[236]

However, a number of investigators brought forth clinical and experimental evidence to cast doubt upon this theory,[237] and more recently (1936), Abel[238] and his associates brought strong evidence to light that

seems to refute this conception. They have called attention to the fact that there is no such thing as 'protoplasmic streaming' within the axis cylinder of the nerves and that the perineural lymphatics pass upward to regional lymph glands and not to the spinal cord. Likewise, there is no possibility for the toxin to pass up through the tissue spaces between the fibers in a nerve bundle. They believe that local tetanus is produced *by direct action of the toxin on the muscles* in the neighborhood of the distributing focus and that all the rest of the toxin is rapidly taken up through the blood capillaries and lymphatics into the blood stream and distributed throughout the body. They believe that there is no 'blood-brain barrier' preventing the passage of toxin into the susceptible central-nervous-system tissue and no barrier between the blood and other susceptible tissues. Animals are killed by the toxin, and at necropsy, the organisms may be found only at the site of inoculation. This is also usually true of human beings who have died of tetanus, although Tizzoni and Cretite [239-40] have recovered tetanus bacilli from the spleen and heart's blood of infected human beings.

C. OTHER CLOSTRIDIA

A description of other commonly found nonpathogenic anaerobic Gram-positive sporulating bacilli present in gas-gangrene and wound infections is given in the chapter on laboratory methods.

2. *Gram-positive Nonsporulating Anaerobic Bacilli*

This group of anaerobic organisms has received but little attention. Prévot [169] lists 33 species, which he divides into 4 groups on the basis of cell morphology. These bacilli have also been studied by Eggerth [241] and by Eggerth and Gagnon,[242] who regularly isolated them from human feces. The most important species from the standpoint of surgical infections is *Bacterium ramosus.*

A. BACTERIUM RAMOSUS

There are numerous reports in the European literature of the isolation of *B. ramosus.* It would seem to be a true pathogen and has been recovered in pure culture as well as mixed cultures from surgical infections. It was first described by Veillon and Zuber in 1898,[243] and has been isolated from mastoiditis, acute and chronic otitis, suppurative pleurisies, lung gangrene, and liver abscesses, as well as from infections arising from lesions of the intestinal and genitourinary tracts. *It may invade the blood* stream and give rise to septicemia. Prévot states that *it is a natural inhabitant of mucous membranes* of man and animals. It has been isolated from sea water.

(1) *Morphology: Bacterium ramosus* is usually a thin rod, straight or slightly curved, but it may be filamentous. Y and V forms are not uncommon. In the filamentous forms, spheroid swelling is sometimes observed. It is nonmotile and nonspore-forming. It is Gram-positive.

(2) *Cultural Characteristics:* Growth on primary isolation may take 2 to 8 days to appear. After several transplants on laboratory media, growth occurs in twenty-four hours. A general turbidity is produced in broth, and a deposit settles out after several days. A moderate amount of gas with a slightly rancid odor is formed. Colonies on the anaerobic blood plate are very small and translucent. There is no zone of hemolysis. This bacillus is a strict anaerobe.

(3) *Toxin Production:* Prévot [169] reports that in filtrates of broth cultures a toxic substance is present that when injected into the muscles of a guinea pig produces spasms followed by death within a few minutes.

(4) *Animal Pathogenicity:* Some strains of this bacillus are reported to be pathogenic for rabbits and guinea pigs. Subcutaneous injection into guinea pigs resulted in abscess formation, cachexia, and death. Intravenous injection into rabbits caused intoxication and death after several days.[244]

3. *Gram-Negative Nonsporulating Anaerobic Bacilli*

The anaerobic Gram-negative nonsporulating bacilli are a large and complex group of organisms. Only a few of the species reported have been studied systematically, and many of the species listed have been created from an incomplete study of a single strain. However, we can distinguish certain broad groups on the basis of morphology, and these have a considerable importance in surgical infections.

A. FUSOBACTERIA

These bacilli may be found around the teeth in the grossly normal oral cavities of man and animals. Fusiform bacilli are found in acute gangrenous and also in chronic infections growing in symbiosis with spirochetes and streptococci, especially the anaerobic nonhemolytic streptococci. This symbiotic growth is the cause of such virulent infections as Vincent's angina, the gangrenous noma in children, the foul-smelling infections of the mouth and jaw that may follow the extraction of teeth, lung gangrene, and lung abscesses. Human bites that become infected with these fusospirochetal organisms represent a particularly serious form of infection, and bones, tendons, and joints may be invaded and destroyed. These organisms have also been found in chronic bronchiectasis, chronic otitis media, and chronic osteomyelitis. Fusospirochetal

infections of the eye have also been reported.[245] There is no clear-cut evidence that the fusobacteria may of themselves cause infections in man or laboratory animals. They are undoubtedly 'opportunist pathogens.'

(1) *Morphology:* These organisms are nonspore-forming rods, usually spindle- or cigar-shaped. Some species have nucleated granules. Motile forms have been described. They are Gram-negative.

(2) *Cultural Characteristics:* The Fusobacteria are obligate anaerobes, grow poorly in or on ordinary media, and require enrichment of the medium with blood, ascitic fluid, or vegetable extracts.[246] The colony type varies. It may be convex and circular, or granular, flat, and rhizoid; gray and translucent, or opaque and brownish.[247] Some strains produce a rancid odor. These bacilli are very sensitive to atmospheric oxygen and exposure to air for four to six hours will kill many freshly isolated strains.[247]

(3) *Classification:* Various attempts have been made to classify the fusiform bacilli on a serological basis,[248-9] but no generally accepted scheme has been evolved.

B. BACTEROIDES

The bacteroides comprise another large group of Gram-negative nonsporulating bacilli. They are indigenous parasites or saprophytes of all the mucous membranes. Prévot [169] and Lewis and Rettger [250] have divided them into subgroups: (1) simple rods and (2) pleomorphic bacilli. The type species for the first group is *Bacteroides fragilis*, and for the second, *Bacteroides necrophorus.*

(1) *Bacteroides fragilis Group:* These simple forms of bacteroides may be found in a wide variety of lesions where the bacterial flora represent contamination by or invasion from the mouth, gastrointestinal, or genitourinary tracts. Altemeier [251] believes that these organisms together with the anaerobic nonhemolytic streptococci are responsible for the foul odor in putrid peritonitis. They may be isolated in pure culture from septicemias and meningitis, as extensions of acute or chronic infections.[252] We have isolated them in pure culture from a large intramural abscess of the abdominal wall, which healed rapidly after surgical drainage, and from fatal cases of multiple liver abscesses, as well as from mixed infections of the peritoneal cavity, rectal and pilonidal abscesses, liver and lung abscesses, and dental infections.

(a) *Morphology.* Although the members of this group are essentially simple rods, they vary considerably in morphology in different species. They may be straight or slightly curved, with blunt or tapered ends, motile or nonmotile, encapsulated or nonencapsulated.

(b) *Cultural Characteristics.* These bacilli are strict anaerobes and most strains require enrichment of the media with body fluids for growth, especially in the primary plantings. In cooked-meat medium, some strains form gas. The production of fetid odor is variable. On blood-agar plates after 48 to 72 hours' incubation in the anaerobic jar, the colonies are small dewdrops and are nonhemolytic. One species, *Bacteroides melaninogenicum*, produces a distinctive colony. This bacillus forms a black pigment from blood and often grows in symbiosis with the anaerobic nonhemolytic streptococcus. For this reason, isolation in pure culture may be difficult.[253]

(c) *Animal Pathogenicity.* Altemeier[251] has reported the production of fatal peritonitis in laboratory animals using pure cultures of *Bacteroides fragilis* and *Bacteroides thetoides*. His strains of *B. melaninogenicum* were relatively avirulent, and he believes that the composite virulence of these strains is much greater than that of any one. Weiss,[254] on the other hand, has recovered strains of *B. melaninogenicum* from human lesions that, if injected within a few days after isolation, were pathogenic for rabbits and mice. He believes that this bacillus is an 'opportunist' pathogen of undeniable importance.

(2) *Bacteroides necrophorus Group* (*Actinomyces necrophorus*): The bacilli belonging to this group are also indigenous to mucous membranes. They are highly pathogenic for man and for animals. In man they have been isolated from septicemias, often fatal, following pharyngeal infections,[255-6] surgery of the intestinal tract,[257] and of the genitourinary tract.[258-9] Smith et al.[252] report 5 cases of meningitis, 2 complicated by brain abscesses, all of which developed from chronic otitis media. These bacilli have also been recovered from suppurative lesions of the mouth, respiratory, gastrointestinal, and genitourinary tracts. Dack and Dragstedt[260] believe that they play an important role in ulcerative colitis. We have isolated them in pure culture from an infected thyroglossal cyst and in association with anaerobic nonhemolytic streptococci from a fulminating cellulitis of the face following the extraction of a tooth, from ulcers of the vagina in a 3½-year-old girl that had persisted for 3 years, and from a chronic draining abdominal sinus following oophorectomy. We have also found them in the mixed bacterial flora of intra-abdominal abscesses, liver abscesses, and peri-rectal abscesses

(a) *Morphology.* These bacilli are highly pleomorphic, and may be coccoid, small, straight rods or long, wavy filaments with occasional branching. The long filaments are characterized by swollen bodies (see Fig. 19). These spheroids may also be extracellular, and in cultures of six to ten days may be the predominating form. In a pure culture, any or all of these forms may be present.



FIG. 19. Pure culture of *B. necrophorus* obtained from a thyroglossal cyst

(b) *Cultural Characteristics.* These pleomorphic bacilli grow well in cooked-meat medium, especially if ascitic or pleural fluid is added. Colonies on blood-agar plates incubated anaerobically are round or rhizoid, gray translucent, sometimes granular, feebly hemolytic or non-hemolytic. Dack [201] believes that *B. funduliformis* and *B. necrophorus* should be considered as a single species, as the cultural differences between the two are no greater than the strain variations in either.

(c) *Toxin Production. Bacteroides necrophorus* forms a powerful exotoxin, lethal to guinea pigs on intravenous injection. When rabbits are injected subcutaneously, an abscess develops that may ulcerate out and then heal, or the animal may die with rapid and progressive emaciation.[202]

(d) *Animal Pathogenicity.* The funduliformis-necrophorus bacilli are a common cause of liver abscesses in cattle and of labial necrosis in sheep.

III. MICROAEROPHILIC BACTERIA

A. MICROAEROPHILIC STREPTOCOCCI

1. *Hemolytic Streptococci*

Microaerophilic hemolytic streptococci are encountered frequently in cultures of surgical infections. During a two-year period in our laboratory, hemolytic streptococci were isolated from 577 infections; 149 strains or 25.7 per cent grew anaerobically only upon primary isolation from the infectious process. They are also found in nose and throat cultures and fecal specimens. In this group are included all beta hemolytic streptococci that grow only under reduced oxygen tension on primary platings of the culture material. The microaerophilic hemolytic streptococcus as a rule adapts itself readily to atmospheric oxygen pressure and will grow on the surface of blood-agar plates under aerobic conditions after one to four transplants on laboratory media. In this group are included also all the strains in which certain individuals of a given strain

will show surface growth under aerobic conditions, but in which the great majority require a lowered oxygen tension for multiplication. There are other strains that show aerobic growth only after forty-eight to seventy-two hours' incubation but that show typical well-developed colonies after twenty-four hours' anaerobic incubation. Many of these strains will multiply aerobically only at room temperature and not at 37° C. All of these types show a decided preference for lack of free oxygen in the environment, and thus may be classified as microaerophilic, which of necessity is a relative and not an absolute term. Some of the strains we have isolated retain their microaerophilic character throughout the course of the infection. In one case of multiple pelvic abscesses developing over a two-year period, the hemolytic streptococcus isolated from the primary drainage of each new abscess grew anaerobically only. After surgical drainage was established, the streptococcus lost its microaerophilic character. We have also observed instances in which in chronic infections the streptococcus, at first aerobic, developed a microaerophilic character as the infection persisted, especially if cultures were taken from the depths of sinus tracts.

It is our opinion that these streptococci do not belong to a different species from the aerobic streptococci, but that often through residence in an environment poor in oxygen, as for example in the intestine or in lymph glands, variants arise that have lost their capacity for normal multiplication in the presence of free oxygen.

The microaerophilic hemolytic streptococcus does not differ in morphology or cultural characteristics (except for the preference for a lowered oxygen tension) from the aerobic strains. We have isolated strains belonging to Lancefield's Group A, B, C, D, F, and G. The Group-A strains tested formed a lysin that dissolved human-plasma clot. The lipase content was similar to that of aerobic varieties.

Microaerophilic hemolytic streptococci are true pathogens. They are the etiological agents in the chronic, undermining, burrowing ulcers described in Chapter XIV. They have been recovered in pure culture from the deep sinuses or undermined margins of many of these ulcers, but owing to the chronic character of this lesion, other pathogens or saprophytes common to infections of long standing are often present on the surface. Microaerophilic hemolytic streptococci have been found in a great variety of surgical infections. We have cultured it from varicose, postradiation, and other types of ulcers; from chest fluids, peritoneal fluids, and bile It was also found in lung, peritoneal, perirectal, and other abscesses, in infected traumatic wounds, and in postoperative wound infections, especially those related to abdominal operations It has been recovered in pure culture as well as associated with other organisms.

Attempts to produce a typical undermining, burrowing ulcer in

laboratory animals have not been successful (mice, guinea pigs, rabbits, and monkeys). Upon subcutaneous or intracutaneous injection, a lesion develops, but it does not take on the distinctive properties of this infection.

2. *Nonhemolytic Streptococci*

Prévot[169] lists the microaerophilic nonhemolytic streptococcus as a definite species with distinctive cultural characteristics. However, although we have isolated strains belonging to this species '*evolutus*,' we have isolated many more strains that differ in their cultural characteristics, notably in the liquefaction of gelatin. It is probable that many microaerophilic variants arise from aerobic strains in envronments of low oxygen tension. The microaerophilic nonhemolytic streptococci do not vary in morphology from aerobic strains. They grow as diplococci, or in short or long chains. On anaerobic blood-agar plates, the colonies may have no effect on the surrounding red blood cells or may produce some green discoloration that develops after the plates have been exposed to the air. The microaerophilic nonhemolytic streptococci do not adapt themselves as readily as the microaerophilic hemolytic streptococci to atmospheric oxygen pressure, and it may require 8 to 10 transplantings before multiplication takes place aerobically. Strains of nonhemolytic streptococci that grow anaerobically only on solid media after 10 transplantings may be assumed to be obligate anaerobes. We have never encountered a gas-forming microaerophilic nonhemolytic streptococcus. It is a normal inhabitant of the respiratory and alimentary tracts and also of the vagina.

Although this organism may be cultured from many types of surgical infections, it has been invariably found associated with the chronic synergistic gangrene described in Chapter xiv. In the gangrenous area it grows in synergism with a hemolytic *Staphylococcus aureus*, but in the subcutaneous tissue of the erythematous area beyond the gangrene, it is present in pure culture. We have observed in cultures from certain of these cases, the streptococcus colonies growing in a halo around staphylococcus colonies on aerobic plates. On the portions of the plate free of staphylococcus colonies, the streptococci did not develop.

In pure culture, the microaerophilic nonhemolytic streptococci isolated from the synergistic gangrene seemed to be entirely nonpathogenic for animals, although it may remain alive in the peritoneum of guinea pigs for at least one month. If it has been growing aerobically before injection into the guinea pigs, it may regain its anaerobic property after a month's duration in the peritoneal cavity. When combined with the staphylococcus with which it is associated in the gangrenous process, it forms a lesion in guinea pigs, rabbits, and dogs that neither can produce

alone. The infection generally goes on to a rather extensive necrosis of the skin, but does not progress in the same way it does in man. The process spontaneously comes to a standstill, and the gangrenous skin sloughs out with subsequent healing of the wound. Filtrates from this organism appear to be nontoxic when injected into animals even when combined with staphylococcal cells. Apparently the symbiotic nature of the infections seem to require the active growth of both of these organisms.

Microaerophilic nonhemolytic streptococci have also been isolated from infections related to the gastrointestinal tract, such as intra-abdominal abscesses and postoperative-wound infections.[161] It has also been recovered from pulmonary gangrene, lung abscesses, and puerperal fever.[262]

B. CLOSTRIDIUM HISTOLYTICUM

In 8 cases of gas gangrene, *C. histolyticum* was found by Weinberg and Séguin[175] who named it 'histolyticus' from the rapidity with which it lysed tissues. MacLennan[176] found it in 10 out of 146 cases of gas gangrene in association with other pathogenic and nonpathogenic clostridia. All of these cases resulted fatally. In the laboratory of the Presbyterian Hospital Unit of the Contaminated Wound Study, it was isolated twice from tissue removed at primary débridement of compound fractures. In both of these cases, the wounds were heavily contaminated with filth, one from manured garden soil, and the other from railroad tracks. In neither case did it persist in the wound following débridement. It would seem to be a relatively uncommon but widely dispersed organism in the soil.[194, 263-4] It has also been isolated occasionally from the human intestinal tract.[265]

(a) *Morphology. Clostridium histolyticum* is a motile Gram-positive rod 0.5 to 0.7 μ by 3.0 to 5.0 μ. It occurs singly and in pairs. The spores are oval, subterminal, swell the rod, and are freely formed (Fig. 20).

(b) *Cultural Characteristics.* The histolyticus bacillus is a microaerophile. On the aerobic plate, the colonies are very small, and no spores are formed under aerobic conditions. On blood-agar plates under anaerobic conditions, the colonies are slightly larger, round, and translucent. A small zone of hemolysis is produced. It grows readily in cooked-meat or brain medium, which are digested and rapidly blackened if iron is present. Milk containing iron wire is also rapidly digested and blackened. Gelatin is liquefied with the production of a port wine color characteristic for this species.[213] No sugars are fermented. In old cultures, there is a deposition of tyrosin crystals.

(c) *Toxin Production.* The histolyticus bacillus produces a powerful proteolytic toxin and hemolysin.[266-8] This is antigenic and a potent antitoxin has been produced. The toxin when injected subcutaneously may

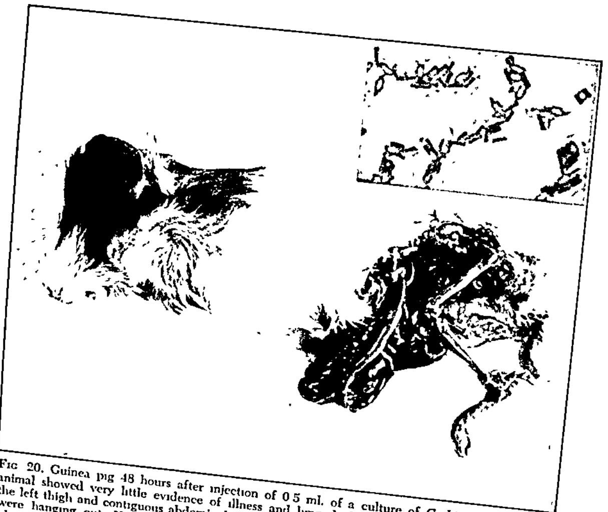


Fig 20. Guinea pig 48 hours after injection of 0 5 ml. of a culture of *C. histolyticum.* The animal showed very little evidence of illness and limped about although the soft tissues of the left thigh and contiguous abdominal wall had been completely liquefied and the intestines were hanging out. He was killed by anaesthesia. *Insert.* *C. histolyticum* from blood plate. × 1500 (From Nelson's *Loose-Leaf Surgery.* Courtesy of Thom[illegible] from blood [illegible]

produce only a redness of the skin and moderate swelling in 2 to 4 days, but intramuscular injection into animals usually results in necrosis of the skin and lysis of the muscle.

Reed, Orr, and Brown[183] report that the production of active fibrinolysin by *C. histolyticum* is comparable to that by *C. welchii.* All of the strains tested lysed coagulated plasma of man, guinea pigs, rabbits, and sheep. This lysin is nonantigenic and is not identical with the proteolytic enzymes of the bacillus. It is noteworthy that cultures of *C. tetani* did not produce this lysin, which was elaborated by the gas-gangrene clostridia tested.

(d) *Animal Pathogenicity.*[108] When cultures of *C. histolyticum* are injected into the thigh muscles of a guinea pig, a profound liquefaction of the soft part, which leaves the bones bare, is produced within 12 to 48 hours. Occasionally, there is no further invasion of tissues, the leg may amputate itself, and the pig recover. In the majority of instances, however, the process extends to the abdominal wall, and evisceration occurs. During this process, the animal may not appear to be acutely ill. Death may occur as a result of extensive hemorrhage or of secondary infection of the soft parts and the peritoneal cavity (Fig. 20).

IV. SPIROCHETES

Spirochetes were among the first micro-organisms to be seen, and were described by van Leeuwenhoek in 1683.[269] Their systematic study, however, dates from the work of Ehrenberg,[270] who in 1833 gave them their generic name and described various forms occurring in water. The term 'spirochete' is a general one for all motile, twisted, flexible, elongated micro-organisms, and includes the true Spirochaeta, the Leptospira, the Cristispira, and the Treponema. With few exceptions, the agents responsible for pathological lesions in man and animals are the Treponema. The 'oral spirochetes,' which play so important a role in the group of infections known collectively as 'fusospirochetal' belong to this group, as does the etiological agent of syphilis, *T. pallidum.*

A. TREPONEMA PALLIDUM

This organism concerns the surgeon only when the syphilitic process necessitates the removal of a degenerated organ, that is, when caseation of a gumma of a testicle has broken through the skin and subjected the part to secondary infection. However, since many inflammatory processes and new growths are frequently confused with the primary, secondary, and tertiary lesions of syphilis, syphilitic processes come within the interest of the surgeon.



Fig. 21. Spirochaeta pallida in India ink preparation. (From Park and Williams, *Pathogenic Microorganisms*, Lea and Febiger, Philadelphia; and Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

The diagnosis of syphilitic infections is bacteriologic and serologic. The bacteriologic diagnosis consists in the demonstration of the organisms under dark-field illumination with the microscope or in stained smears. Although these spirochetes have been grown in pure and mixed cultures by various workers,[271-2] cultivation *in vitro* is not practical for diagnostic purposes. Serologically, the Kahn and Wassermann tests are used for diagnosis.

B. 'Commensal Spirochetes'

Under this heading are included the Treponema, which play a part in fusospirochetal disease (see under Fusobacteria). These spirochetes were described by Miller,[273] who in 1890 offered the first comprehensive description of organisms found in the mouth. This group of Treponema are natural inhabitants of the mucous membranes of the mouth and of the anogenital regions. Many forms have been described and listed as 'species' from observation of their morphology in body exudates, in pus from infectious processes, and in mixed cultures, but only a few types have been obtained in pure culture. Noguchi[274] was the first to devise a method whereby they could be isolated in pure culture. Rosebury,[275-6] has modified and improved this technic and brought *in vitro* cultivation within the realm of practicability for bacteriologists who are not specialists in this field. His use of dark-field microphotographic motion pictures has also contributed greatly to our knowledge of the morphology and the type of motility of the different species.[277]

1. *Classification and Morphology*

The four species most commonly found in the human mouth and in fusospirochetal infections are *T. vincenti, T. buccale, T. microdentium,*

and *T. macrodentium*.[273, 277-8] *Treponema vincenti* is a large delicate form with open spirals; *T. buccale* is large and coarse with a double contour and with open spirals; *T. macrodentium* is a large thin spirochete with regular spirals more tightly wound than those of *T. vincenti* or *T. buccale*, whereas *T. microdentium* is a smaller form with tightly wound spirals. *Treponema microdentium* is very active, and the coils are not altered in locomotion (see Fig. 22). These spirochetes are all strict anaerobes and rapidly lose their motility and die if material from human infections is allowed to cool or is exposed to the air.

2. *Pathogenicity*

There is no proof that any of these mouth spirochetes can of themselves produce an infectious process in man or in animals.

In human fusospirochetal infections all four types are seen in surface lesions, but in deeper lesions, only *T. microdentium* is regularly found. In experimental infections produced in guinea pigs or rabbits by the inoculation of material obtained directly from human spirochetal infections, *T. vincenti* and *T. buccale* tend to disappear, whereas *T. macrodentium* and *T. microdentium* persist.[278-80] *T. microdentium* is usually the predominant spirochete, and this is the type found to be essential in the production of experimental fusospirochetal infection when pure cultures of spirochetes, fusiform bacilli, vibrios and anaerobic nonhemolytic streptococci are combined. Most workers,[278-81] agree that for the production of a gangrenous process experimentally, all four organisms must be

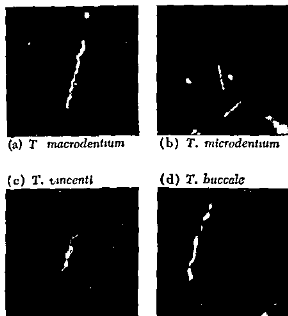


FIG 22. Four Spirochetes (Courtesy of Dr Theodor Rosebury)

present. Spirochetes or fusiform bacilli together with the anaerobic nonhemolytic streptococcus may produce a lesion, but this lesion lacks the characteristics of a typical fusospirochetal infection, which is dependent on the symbiotic action of these organisms. This is also true clinically—the most destructive and virulent lesions contain a mixture of these four organisms.

V. FUNGI

Henrici [282] divides the mycotic infections into two groups, the superficial and deep-seated mycoses. The superficial mycosis (Dermatomycoses, Moniliases) are of common occurrence, self-limited, and rarely fatal. The deep-seated mycoses (Actinomycoses, Debaromycoses, Blastomycoses, and so on) are rare. They cause a progressive disease that is often fatal. It is this latter group (the deep-seated mycoses) that is of interest to the surgeon, not only because the lesions may at times simulate infections that require surgical therapy, but also because in some instances, early removal of local lesions before metastatic involvement has occurred may eradicate the disease. Surgical intervention is also required for the drainage of abscesses or necrotic lymph nodes.

A. ACTINOMYCES

1. *Actinomyces israeli*

In 1887, Bollinger [283] gave the first accurate description of the 'ray fungus' causing lumpy jaw in cattle and in 1878, Israel [284] isolated a similar organism from a human infection. The *A. israeli* (humanis and bovis varieties) produces a chronic progressive infection in man, which usually affects the jaw and tongue and at times the lung, brain, liver, and paraintestinal tissues. From these sites it spreads either by direct invasion of neighboring tissues or cavities or through the blood and lymph streams to form metastatic abscesses. Topley and Wilson [285] give the distribution of 455 actinomycotic lesions in man as follows: head and neck 51.5 per cent, tongue 3.5 per cent, abdomen 21.8 per cent, thorax 14.3 per cent, skin 2.4 per cent, other situations and doubtful 6.6 per cent. Heart lesions have been described.[286] Blair [287] and Fuller and Wood [288] have reported cases of primary actinomycosis of the stomach. These lesions are characterized by the development of so-called 'sulphur granules' in the pus or discharge from sinuses. These are really colonies of the actinomycete, a mat of tangled filaments often with clubbed ends. These granules may be crushed between cover glasses and examined microscopically in making a diagnosis of the lesion. In tissue sections stained with hematoxylin and eosin, the colonies appear as dense



FIG. 23. Colony of actinomyces in tissue showing the typical arrangement which gives it the name of 'ray fungus.' (Courtesy of Dr. A. P. Stout)

blue masses with a red fringe formed by the acidophilic clubbed ends. It is this picture that has given them the name 'ray fungi' (Fig. 23)

(a) *Morphology. Actinomyces israeli* is a branching, filamentous organism. The threads may be continuous or segmented. The tips may show swollen or club-shaped ends. Often the filaments are broken up into short rods, which bend at sharp angles. Variations may occur in culture media, and the short bacillary forms may predominate. These resemble diphtheroids or cocco-bacilli, and there may be little or no branching. This actinomyces is nonspore-forming and nonmotile. It is Gram-positive and nonacid-fast (Fig. 24).

(b) *Cultural Characteristics.* The *A. israeli* grows best under anaerobic conditions, especially in the presence of 5 per cent carbon dioxide Some strains are obligate anaerobes, but others are microaerophilic. In liquid medium under anaerobic conditions, there is a growth of tiny colonies or granules forming a deposit in the tube. On solid medium, the colonies are nonhemolytic, convex, and opaque, from 1 to 3 mm. in diameter after 5 to 6 days' incubation. The 'rough' forms often come away in one piece

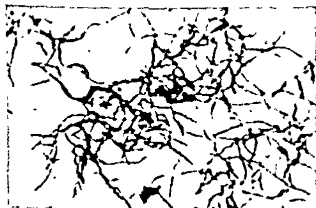


FIG. 24 Actinomyces. Culture of *Actinomyces israeli* obtained from a lesion of the right lower quadrant of the abdomen.

and are difficult to emulsify, whereas the 'smooth' forms are soft and easily broken up. These resemble colonies of white micrococci or diphtheroids.[289]

(c) *Animal Pathogenicity. Actinomyces israeli* (humanis and bovis) is the causative agent of true actinomycosis in man and in cattle. When pure cultures of strains isolated from these infections are used, it is difficult to reproduce the disease in guinea pigs or rabbits by a single injection. Repeated injections at intervals have resulted in progressive and fatal actinomycosis in these animals, which closely resembles the natural disease.[290]

B. CRYPTOCOCCUS NEOFORMANS (CRYPTOCOCCUS HOMINIS, TORULA HISTOLYTICA, DEBAROMYCES NEOFORMANS)

This yeast is the causative agent of the European type of blastomycosis, first described by Busse[291] and Buschke.[292] It is a systemic disease, the organisms entering the body through the lungs or the intestinal or genital tracts, and then producing metastatic abscesses in the subcutaneous tissues, in the retroperitoneal tissue, and in the bones and joints. It runs an essentially chronic course and usually ends fatally. These cryptococci may be found on normal skin and in the alimentary tract.

1. *Morphology*

The cells vary in size from minute rounded objects scarcely a μ in diameter to large hyaline bodies 40 to 50 μ in diameter. The organisms are usually spherical but may be somewhat ovoid. This yeast forms a gelatinous capsule of varying size. In tissue sections, the capsule appears as an unstained zone, which may be more than twice the diameter of the cell itself. The capsule may be easily seen in sodium hydroxide mounts from infected tissue. If culture material is diluted with India ink and then drawn out in a thin film on a glass slide, the capsules stand out clearly (Fig. 25).

2. *Cultural Characteristics*

This organism reproduces by budding, and forms no mycelium. It may be differentiated in this way from the Monilia, which regularly form mycelium on corn-meal agar. On maltose or honey agar, the colonies of the *Cryptococcus neoformans* are moist and smooth. The cream-colored growth darkens with age to a deep brown. On blood-agar plates young colonies may be small, transparent, and mucoid in character, or they may resemble the colonies of *Staphylococcus aureus*.

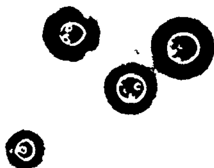


FIG 25. *Cryptococcus hominis*. Showing the capsules of *Cryptococcus hominis* stained with India ink. (Courtesy of Dr. Rhoda Benham)

3. *Animal Pathogenicity*

Benham[293] reports that freshly isolated strains have a 'variable but slight virulence' for laboratory animals. Mice and rats are more susceptible than guinea pigs or rabbits.

C. BLASTOMYCES DERMATIDITIS

The 'American' type of blastomycosis is a clinical entity, distinct from the 'European' type, which is caused by the *Crytococcus neoformans*. This 'American' type first described by Gilchrist in 1896[294] is caused by *Blastomyces dermatiditis*. It is essentially a disease of the skin and subcutaneous tissues starting as a papulopustule on the hands, face, or neck, which gradually penetrates the deeper tissues with the formation of low-grade abscesses. When the disease remains confined to these tissues, the course is chronic but recovery eventually occurs. The systemic form of the disease may result from a spread to the neighboring lymph glands, with multiple metastatic abscesses throughout the body, both in the



FIG. 26. Blastomycosis Blastomycosis in the tissues following animal inoculation (Courtesy of Dr Rhoda Benham)

subcutaneous tissues and in the viscera and muscles. The primary focus of the infection is in the lungs in some cases, and at times it is difficult to differentiate such infections from tuberculosis. When the disease becomes generalized, the outcome is usually fatal. Colonna and Gucker[295] in a review of 243 proved and presumptive cases of generalized infection report that 50 per cent had bone and joint involvement. The source of the infection is unknown. The majority of cases reported have occurred in this country. It is endemic in the neighborhood of Chicago.

1. *Morphology*[296]

The organisms appear in the tissues or in discharges from the infection as round, thick-walled budding cells from 8 to 20 μ in diameter. They may be seen best when such material is suspended in 10 per cent sodium hydroxide. As a rule, the cells are highly granular. The buds form one at a time and have a thinner wall than the parent cell.

2. *Cultural Characteristics*

The colony form is variable. Henrici[297] describes three types on maltose agar—'mealy,' 'prickly,' and 'cottony.' In the 'mealy' type, the budding cells predominate. With the development of mycelium after prolonged incubation or after several transfers on laboratory media, the spiny elevations that appear on the surface are responsible for the 'prickly' appearance. The third or 'cottony' type is characteristic of strains that have been grown on laboratory media for some time. The cottony appearance is due to abundant aerial mycelia. On blood agar, growth is usually of the mealy type. This fungus grows best at room temperature but will develop at 37°.

3. *Animal Pathogenicity*

Forshay and Madden[298] report a spontaneous generalized infection in a dog. When this strain was compared with strains isolated from human infections, no differences were discernible. Benham[299] states that heavy subcutaneous inoculation into dogs or monkeys results in a local suppurating granuloma with metastatic lesions in the lungs and other viscera (Fig. 26). Spring[300] reports mice to be more susceptible than guinea pigs.

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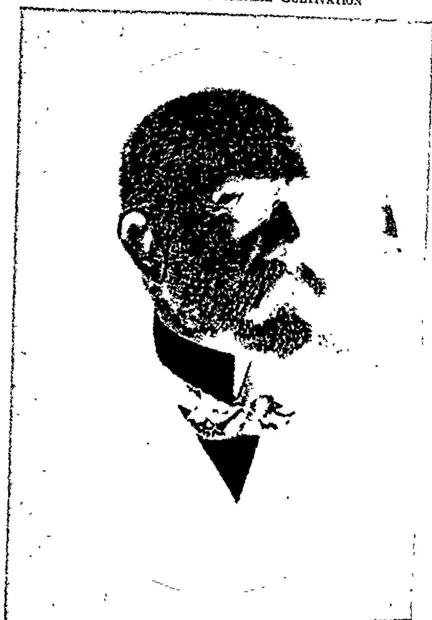


FIG. 1. KOCH. After Pasteur, Robert Koch, the greatest of the early bacteriologists, perfected the isolation of bacteria by the growth of colonies on solid media.

food substances on the surface rather than the tissue of the host. When these surfaces are broken either by the specific action of a pathogenic organism or by some mechanical force, all the organisms present may invade for a certain distance; but only those with inherent capacity for penetration and survival against the defensive forces of the body may be expected to be found at any great distance from the point of entrance. It has frequently been the custom, when examinations are made of material from the mouth, nose, or alimentary tract, to lay

particular emphasis on the 'predominating type' of organism present; but it is obvious that the 'predominating type' is often by no means the significant type. It is important, therefore, for the bacteriologist to know certain of the clinical points in the history of the case from which the material is taken, for in many cases the significant organism may be anticipated to some extent and the proper culture medium used.

It is known that some organisms will grow in the simplest media, whereas others require certain unusual elements found in the animal body but not present in the ordinary laboratory media. Some of these characteristics have been mentioned in the preceding chapter, and it is obvious that there may be still other organisms growing within the animal body that have not yet been cultivated outside it because the proper medium has not yet been devised.

These facts are mentioned in order that there may be a rationale for the collection and examination of material.

We cannot overemphasize the fact that any analysis of the bacteriologic flora of a surgical infection failing to take into account the anaerobic as well as the aerobic bacteria is inadequate. Improvements in media and technics have made the isolation of anaerobic organisms no more difficult than that of many of the aerobic organisms. The only radically different procedure is the removal of atmospheric oxygen from the bacterial environment, and this can easily be accomplished by use of an anaerobic jar (see section on Special Technics).

This chapter is not intended to be a complete manual of bacteriologic technics. It represents a résumé of technics that have been used and found satisfactory in the study of surgical infections in the Surgical Bacteriologic Research Laboratory of the Presbyterian Hospital in New York City. We have attempted to outline practical procedures for diagnostic work rather than ideal methods for special research problems. The material to be covered has been divided into the following sections:

Collection of Specimens
Methods for Bacteriologic Examination
(a) Procedures for Preliminary Identification
(b) Procedures for Final Identification of Culturally Pure Strains
Guide to Media
Preparation of Media
Stains, Indicators, and Special Technics

I. COLLECTION OF SPECIMENS

It is primarily the responsibility of the surgeon to see that adequate material is sent to the bacteriologic laboratory. Sufficient material

should be collected to permit direct microscopic examination by stained smears or dark-field preparations and the inoculation of several different kinds of media. It is important that the culture be representative of the lesion. If swab specimens are taken, such specimens should be made from the most active part of the infection, that is, from the depths of sinus tracts as well as from the whole surface or representative areas of extensive lesions; from the peritoneal fluid around the inflamed or gangrenous appendix rather than from the fluid that escapes as the peritoneal cavity is opened. In closed abscess cavities, random sampling is sufficient. At times it is advisable to send all the tissue removed at operation to the laboratory. The material may then be dissected under the surgeon's direction and cultures taken from the deeper as well as the exposed parts of the infection. This is often of paramount importance in determining the etiological agent in mixed infections.

It is extremely important that the material obtained should not be contaminated by organisms that are on the surface but that have no part in the infection. In living subjects, whenever possible, the skin surface should be cleaned and treated with iodine, which is the best of the so-called 'skin antiseptics,' before any material is taken for culture. At necropsy, the skin may be treated in the same way or painted with pure carbolic acid. A convenient and effective method of sterilizing the surface of an organ is to sear it with a hot spatula and open the organ with a sterile instrument, care being taken to prevent tissue fluids from contaminating the seared area. It should be borne in mind that changes take place in the body after death, particularly by the action of micro-organisms, and cultures of the blood or tissue of the cadaver must be considered in this light and their significance discounted according to the time that has elapsed between death and the obtaining of the culture material. Blood cultures from the spleen or the brachial angle are more likely to be free from contamination than is the heart's blood.

All specimens should be adequately labeled. The source of the material and, if possible, the nature of the infection should be indicated. If the patient has received sulfonamide or penicillin therapy, this should also be indicated so that the proper inactivators may be added to the culture medium.

II. METHODS FOR BACTERIOLOGIC EXAMINATION

The surgeon is entitled to as complete a report as possible within 24 hours after the receipt of the culture by the laboratory. For this reason, we have divided this section into two parts: (A) Procedures for

Preliminary Identification; and (B) Procedures for Final Identification of Culturally Pure Strains.

A. PROCEDURES FOR PRELIMINARY IDENTIFICATION

A preliminary identification of many of the bacteria can be made on the basis of their morphology—size, shape, and staining reactions—as seen on direct examination of the material; on the basis of their morphology in liquid media; and on their colony characteristics—size, contour, and texture—on blood agar plates (see Chapter IX). We have also indicated certain tests of diagnostic import that may safely be made before the organisms have been proved to be culturally pure strains. Such tests give additional information concerning the potential pathogenicity of the organisms. Preliminary tests for penicillin sensitivity may also be made on the direct platings of the culture material (see section on special technics, p. 337).

1. *Direct Examination*

Direct examination of the fresh culture material by means of smears or films on glass slides will give much valuable information. A Gram-stained preparation (see section on stains, p. 331) will show the number, type, and relative distribution of the bacteria in the lesion. Not only do the results of such an examination often enable the bacteriologist to make primary plantings on appropriate diagnostic media, but also the number of bacteria per microscopic field, the presence or absence of phagocytic cells, and the proportion of intracellular to extracellular organisms are often of definite prognostic value. All material from human-bite infections, from pleural infusions, and from abscesses of the lungs, neck, jaw, and mouth should be examined microscopically in a 'dark-field' preparation for the presence of spirochetes and fusiform bacilli. A smear stained with crystal violet is also required. Such material should be examined immediately after removal from the body, since spirochetes lose their motility at room or refrigerator temperatures. Material from suspected tubercular infections should be examined for acid-fast bacilli in a Ziehl-Nielson stained preparation (see section on stains, p. 331). If a fungus is suspected, the tissue should be digested on the glass slide with 20 per cent potassium hydroxide. Digestion takes less time and is more complete if the slide is warmed gently over a flame before the cover slip is applied. In the absence of organized tissue elements, the fungi can be more easily recognized. When the clinical picture suggests an actinomycotic infection, the specimen should be diluted with saline and a search made for the characteristic 'sulfur' granules. When such granules are found,

they should be transferred to a slide, crushed under a cover slip, and examined microscopically.

Direct examination of tissue is also of great importance, for organisms may be found in the tissues when they cannot be demonstrated in the exudate. This is particularly true of tuberculous material. Furthermore, at necropsy such examinations reveal *not only the morphology of the* organism but also its interaction with the fixed as well as the movable cellular elements of the body. Gram-positive organisms may be brought out very well by certain modifications of the Gram-stain technic especially adapted to tissue, such as the Gram-Weigert method given in the section on *stains. Gram-negative organisms are best brought out* by a general stain including both Gram-positive and Gram-negative bacteria, such as methylene blue, or, better still, strong safranin, if photographs are desired. The Levaditi stain may also be used, but this requires special preliminary treatment of the tissue, particularly in the staining of organisms that do not take the usual aniline dyes, such as the spirochetes or spirilla. For the technic of these procedures, the reader is referred to special treatises on laboratory methods.

2. *Inoculation of Media and Conditions of Incubation*

The ideal method entails the inoculation of the media at the bedside of the patient or in the operating room. Since this is not always practical in routine work, specimens to be cultured should be kept in the refrigerator until sent to the laboratory. A few drops of broth in the tube containing the swab cultures will eliminate the possibility of drying. All material should be studied and planted immediately after receipt in the laboratory.

Both fluid media and solid media are used. Fluid media permit enrichment of the bacterial cultures, but *no separation of the different morphological types.* No idea of the predominant pathogenic organism in the lesion can be formed from an examination of cultures that have been incubated for 24 hours or longer. In fact, an entirely wrong impression may be gained since certain bacteria overgrow other less *rapidly multiplying strains. Solid media* in flat plates permit the isolation of individual species in pure culture. At times, however, the number of organisms present in the original material is too few for growth of any or all bacteriologic forms on the primary *plates, and such bacteria must be recovered from* the subsequent plating of the fluid media.

A. FLUID MEDIA

All cultures should be planted either in duplicate in fluid media designed for the growth of *aerobic or of* anaerobic organisms, or in a medium in which both will multiply. Chopped heart medium (*Medium*

#3) is satisfactory for the growth of both aerobes and anaerobes, if it is boiled for five minutes and then cooled rapidly to room temperature immediately prior to seeding. It is the medium of preference in this laboratory. Not only do the metabolic processes of the aerobic organisms use up the free oxygen in the medium, but the beef-heart muscle contains unsaturated fatty acids and glutathione that aid in the establishment of the proper oxidation-reduction potential for the growth of the anaerobic bacteria. We have found it desirable to incubate the cultures in the chopped heart medium in an anaerobic jar, since this eliminates the risk of losing organisms requiring very strict anaerobic conditions for growth, especially if such organisms are present in very small numbers. Strictly anaerobic conditions are not established quickly enough to prevent growth of obligate aerobic organisms. If a simple infusion or buffered broth (Media #2, #1) is used for growth of aerobic organisms, *the culture material must also be planted in recently boiled* cooked heart medium (Medium #3). Thioglycollate medium (Medium #6) or other semisolid or fluid media may be substituted for the cooked meat if there is special indication for their use.

If the patient has been receiving antibacterial therapy, the proper inactivating agent must be added to the culture media. Para-aminobenzoic acid (5 mgm. per 100 cc. of culture medium) must be added to neutralize any sulfonamide drug present, and a preparation containing penicillinase to inhibit the action of any penicillin that may be carried over into the culture medium.

B. SOLID MEDIA

(1) *Blood-Agar Plates (Medium #15a)*: We see no reason why plain agar plates should ever be used for routine primary platings. The hemolytic activity of the organisms is often of diagnostic importance. Moreover, certain bacteria will multiply only in the presence of body fluids. Duplicate blood-agar plates should be seeded, one for aerobic and the other for anaerobic incubation (see section on anaerobic technics). To ensure the development of discrete colonies, the following technic is used: the upper half of the plate is streaked with the swab, a piece of tissue or a drop of the material as received. The lower half is inoculated by going over the last streak on the upper half with a neochrome ribbon spreader and then continuing the spread over the remainder of the plate. If the organisms in the original material are few in number, they will appear as isolated colonies on the upper half of the plate, but if the material contains many bacteria, the upper half may show almost confluent growth, while the lower half will show discrete and well-differentiated colonies. These may then be fished for the isolation of the organisms in pure culture.

The penicillin sensitivity of the organisms in the culture m tested by placing filter-paper squares moistened with penicillin so (2 units per cc.) on the part of the plate with the heaviest ino (see section on special technics, p. 337).

(2) *Special Diagnostic Media:* The examination of the direct smea location of the infection, or the results of recent bacteriologic e nations of the lesion often make it advisable to use certain s diagnostic media in the preliminary plantings. If the direct shows Gram-negative bacilli or if the lesion has arisen from or is ject to contamination from the intestinal or genitourinary tract cultures should be plated on eosin-methylene blue agar (Medium This will make possible the separation of lactose and nonlactos menting bacilli. Plain agar plates may be used for the recogniti the characteristic greening due to the Pseudomonas. Chocolate (Medium #20) should be used for the Neisseria, and such plates sl be incubated under increased CO_2 pressure. If *in vitro* cultivati the tubercle bacillus is desired, the material should be planted on of Petroff's (Medium #24) or Petragnani's (Medium #25) me If there is reason to suspect *Corynebacterium diphtheriae*, Loe serum medium slants should be inoculated (Medium #19). Cul for fungi should be plated on maltose agar (Medium #32) or h agar (Medium #33). If the morphology of the bacteria seen in smear is suggestive of anaerobic Gram-negative bacilli, direct pla should be made on special veal heart agar (Medium #16). If cul tion of spirochetes is desirable, Hiss serum water (Medium #11) sh be used.

3. *Treatment of Primary Cultures*

A. EXAMINATION OF THE DIRECT PLATES

(1) *Aerobic Plate:* The primary aerobic plates should be examined the aid of a magnifying glass or under a plate microscope after hours' incubation at 37° C. All the morphologically different colo should be ringed with a waxed pencil on the under side of the P dish and numbered. One portion of each colony should be transfe to a drop of saline on a glass slide for a Gram-stained preparation, another portion to 0 2 per cent dextrose cooked meat medium (Medi #3) for isolation of the strain in pure culture. The Gram-stained pre ration is examined under the microscope, and the morphology of bacterial cells—size, form, and staining reaction—noted.

Certain diagnostic tests may be carried out at this time. If a co lation of cell morphology and colonial form is indicative of staphy cocci or other micrococci, the coagulase production and mannitol f

#10) as the diagnostic reactions in these media (stormy fermentation and production of acrolein respectively) develop in the presence of contaminating organisms. Any spreading colonies appearing on the anaerobic plate with cell morphology different from spreading colonies on the aerobic plate should be fished from the advancing margins as far as possible from underlying colonies. If such spreaders are present, particular care should be exercised in fishing all the colonial types, even though subsequent examination will show a duplication of strains, since on further incubation, the entire plate may be covered with a film of growth. Many of the colonies of anaerobic organisms are pin point in size after 24 hours' incubation, and attempts to use the same colony for Gram-stained preparation and inoculation into cooked meat medium are not successful. Unless the clinical condition of the patient makes it desirable, the direct anaerobic plate should not be examined until after a further 48 hours' incubation in the anaerobic jar. The examination of the anaerobic plate at this time may reveal colonies not visible after 24 hours' incubation. This applies chiefly to the anaerobic nonsporulating bacilli and the anaerobic cocci. All colonial forms should be examined in Gram-stained preparations and any morphological types not represented in the 24-hour fishings should be transferred into recently boiled 0.2 per cent dextrose cooked meat medium.

The direct aerobic or anaerobic plates cannot be discarded until all the organisms with different colonial morphology have been isolated in pure culture.

B. EXAMINATION OF THE DIRECT PLANTINGS IN FLUID MEDIA

(1) *Media Not Permitting the Growth of Anaerobic Organisms:* A Gram-stained smear of the culture is made on a slide after 24 hours' incubation. The morphology of all bacterial forms is noted. The culture is then streaked on a blood-agar plate (Medium #15a) for aerobic incubation. The use of a differential medium other than blood agar is based on a correlation of the cell morphology in the Gram stain and the results of the examination of the direct aerobic plate (see Guide to Media, p. 319). A second examination of this aerobic culture is made after 48 hours' incubation, and further platings are required if such an examination reveals morphological forms not present after 24 hours' incubation, or when platings of the 24-hour culture did not result in the recovery from such plates of all morphological forms seen in the Gram-stained preparation. The examination of these platings must follow the outline for the direct aerobic platings noted above.

(2) *Media Permitting the Growth of Anaerobic and Aerobic Organisms (Cooked Heart Medium, Thioglycollate Medium, Brain Medium):* The

presence of odor and/or gas is noted. A Gram-stained smear is made on a slide after 24 and 48 hours' incubation, and all bacterial forms seen under the microscope are charted. Platings are made on duplicate blood-agar plates for aerobic and anaerobic incubation and on special media as indicated by the bacterial morphology (see Guide to Media, p. 319). The examination of these platings must follow the outline for the direct aerobic and anaerobic plates. It must be emphasized that many of the anaerobic bacteria grow slowly, and examination of the 24-hour cultures and of platings made at this time often fail to reveal forms seen on subsequent examinations. All cultures should be kept for at least 7 to 10 days before being discarded, and then only if all morphological forms seen in stained preparations have been recovered in pure culture.

C. SPECIAL TESTS

(1) *Stormy Fermentation of Milk by C. welchii:* If large, thick Gram-positive bacilli morphologically compatible with *C. welchii* are seen on smear, and if this is accompanied by vigorous gas production in the culture medium, a presumptive test for the presence of *C. welchii* is indicated. Iron milk (Medium #12) is inoculated with 0.25 cc. of the mixed culture and incubated aerobically. If *C. welchii* is present, stormy fermentation usually occurs within 5 to 6 hours. The synergistic action of certain bacteria may simulate this stormy fermentation, but this occurs too rarely to invalidate this reaction as a presumptive test for *C. welchii.*

(2) *Heat-Resistance Test.* Since rapid isolation of pathogenic anaerobic spore-forming bacilli is of the utmost importance, the following test should be made after 48 hours' incubation of the fluid anaerobic culture: 0.5 cc. of the mixed culture is transferred to a tube of 0.2 per cent dextrose cooked meat medium. The tube above the level of the broth is well flamed before the stopper is replaced. The tube is then immersed in boiling water for one minute. The level of the water should be well above the level of the culture medium. After 48 hours' incubation at 37° C., the heated culture is examined in a Gram-stained preparation, and if organisms are present, it is plated on blood agar for aerobic and anaerobic incubation. Incubation of heated tubes must be continued for one week at least before being discarded as negative since heat-damaged spores may germinate slowly.

The aerobic plate made from the heated culture should be examined after 24 hours' incubation. The colonies of aerobic spore-forming bacilli are usually well developed at this time, but colonies of the microaerophilic strains, *C. histolyticum* and *C. tertium,* may not be visible

until the plates have been incubated for 48 hours at 37° C. The anaerobic plates should be incubated for 48 hours before being examined, unless the clinical nature of the infection makes rapid isolation imperative. Comparison of the aerobic and anaerobic plates will show whether the flora is a mixture of aerobic and anaerobic sporulating organisms or whether anaerobic organisms only are present. If the flora is mixed, we have found it useful to incubate the anaerobic plate aerobically for a few hours. The colonies of the aerobic bacilli will develop characteristic morphology, and the fishing of different colonial forms for staining and culturing is greatly facilitated.

(3) *Selective Heating:* Spores of certain of the clostridial species differ in their resistance to heat. Therefore, it is possible at times to separate the more heat-resistant by differential heating. A series of 0.2 per cent dextrose cooked meat medium tubes (Medium #3) is inoculated with 0.5 cc. of the mixed culture. One tube is heated in a water bath at 80° C., another at 85° C., a third at 90° C., and a fourth at 95° C. for 20 minutes. The tubes are then incubated at 37° C. for 48 hours. A stained preparation is made and the tubes showing growth are plated in duplicate on blood-agar plates for aerobic and anaerobic incubation. Further treatment follows the outline described in the preceding paragraph.

Since *C. welchii* rarely forms spores in the usual laboratory media,[7] the failure to recover it from heated cultures does not exclude its presence. However, it usually can be counted on to survive 80° C. for 20 minutes. The typical colony formation on direct and secondary blood plates will usually be sufficient for the discovery of this organism.

4. *Isolation of Pure Cultures*

The examination of the primary and secondary platings having resulted in the transfer into cooked meat medium of all the morphologically different colonial forms, the purity of these cultures must be established before final identification tests are made. Cultures of the majority of aerobic organisms and those of the anaerobic Gram-positive sporulating bacilli usually show abundant growth after 24 hours' incubation. Gram-stained smears should be examined microscopically, and the cultures should be replated. Cultures of colonies fished from the aerobic plates need be plated only for aerobic incubation. Those representing colonies fished from the anaerobic plates must be plated in duplicate for aerobic as well as anaerobic incubation. If only one morphological type is present in the stained preparations and only one colony type with a similar cell morphology is present on the replatings, these strains may be assumed to be culturally pure, and fishings may

be made from the replates into the media necessary for the final identification tests. All cultures in the cooked meat medium and all platings should be re-examined after 48 hours' incubation. Replatings and refishings are indicated if additional morphological forms are present.

The same procedures are necessary for the more slowly growing organisms, the anaerobic cocci, and the anaerobic nonsporulating bacilli. As soon as growth is evident (48 to 72 hours), the cultures should be Gram-stained and replated for aerobic and anaerobic incubation. These plates should be incubated for 48 to 72 hours before being examined. It has been our experience that no time is saved by attempts to rush the isolation of slowly growing organisms. Platings of cultures showing relatively few organisms on smear or the fishing of tiny poorly differentiated colonies results in a waste of time and materials, for in many instances the procedures must be repeated.

No cultures made from the primary and secondary platings can be discarded until all morphological forms seen in the Gram-stained preparations have been isolated in pure culture.

Spore-bearing bacilli can be separated from nonsporulating organisms by heating the mixed cultures at a temperature that destroys all vegetative forms. These methods have been described previously under the treatment of the primary anaerobic culture in fluid media.

When Proteus is present in cultures, the spreading colonies may swarm over the entire plate. Proteus itself may usually be isolated in pure culture by picking material from the advancing margin of the spread, but such cultures must be plated on a medium inhibiting this spreading before the other strains can be isolated. We have found 6 per cent blood-agar plates (Medium #15c) very satisfactory.

In certain instances, particularly with the aerobic Gram-negative bacilli, similar cell morphology and similar colony morphology on blood-agar plates may not mean that the strain is culturally pure. Additional media must be used that reveal differential biologic characteristics. The use of eosin-methylene blue agar (Medium #26) will separate the lactose from the nonlactose-fermenting bacilli. Colonies of *Escherichia coli* are deep purple and have a greenish metallic sheen. The lactose-fermenting properties of the Aerobacters may be masked by the production of mucus. This mucoid colony is in itself a differential characteristic. Colonies of the typhoid-dysentery group are small and pale grayish blue. S-S agar (Medium #27) is an excellent medium for differentiating the colonies of the typhoid-dysentery group from the other coliform bacilli. The former appear as colorless colonies, the latter pink.

At times, platings from a seemingly pure culture result in a difference of colony types on the blood-agar plate, that is, smooth (S) colonies, round, convex, and shining, and rough (R) colonies, irregular, flattened,

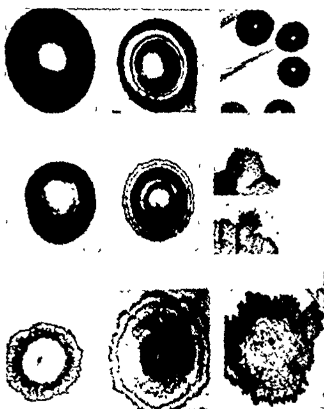


FIG. 2. Smooth and rough forms of hemolytic streptococci. (Courtesy of Dr Gladys Hobby)

and wrinkled. This dissociation is more frequent in strains cultivated *in vitro* for a considerable period. However, avirulent degraded forms with rough colonies may be cultured from infections in which the organisms are not playing a primary etiological role. The organisms in the smooth and rough colonies differ from one another in other characteristics, some of which are considered to be of deep-seated biologic import. Jordan and Burrows [3] outline these differences as follows:

Smooth (S) Type	*Rough (R) Type*
1. Broth cultures uniformly turbid.	1. Sediment deposited in broth cultures; supernatant fluid clear
2. Suspensions in salt solution (0.85 per cent NaCl) stable, remain cloudy	2. Suspensions in salt solution clump spontaneously and settle out
3 Flagellated species usually motile	3. Motility reduced or absent
4. Capsulated species show capsules	4. Capsules absent
5. Somatic and flagellar or type specific antigens present, flocculent agglutination	5. Only somatic antigens present, agglutination granular in type
6. Pathogenic species generally virulent	6. Virulence greatly reduced or absent
7. Biochemically active	7. Biochemical activity reduced
8. 'Normal Morphology'	8. Tendency toward abnormal forms

These differences hold true as a general rule. However, the colonies of hemolytic streptococci are termed 'mucoid' or 'matt' for the 'normal' virulent forms, and 'glossy' for the degraded avirulent types. Various types of Group A colonies are shown in Figure 2. Jordan and Burrows[3] also point out that the 'normal' virulent form of the anthrax bacillus is the rough form and the avirulent form is the smooth type.

B. PROCEDURES FOR FINAL IDENTIFICATION OF CULTURALLY PURE STRAINS

The isolation of culturally pure strains has resulted in a knowledge of the oxygen requirements for growth, the characteristic cell morphology, the type of growth in liquid media, the characteristic colonial form, and certain biochemical activities, such as the production of hemolysin, pigment formation, the fermentation of lactose, and so on. With this information at hand, final tests for identification may be carried out.

1. *Aerobic Bacteria*

A. AEROBIC COCCI

(1) *Gram-Positive Cocci:*

(a) *Streptococci.* The aerobic streptococci are divided into three groups on the basis of the action on red blood cells incorporated in blood-agar plates (Medium #15a). It is not necessary to use pour plates. Undercutting of the aerobic blood plate will show the effect of deep colonies. The final reading of the hemolytic activity is made under a low-power microscope after the plate has been incubated for 48 hours at 37° C, and refrigerated for an additional 24 hours.

Differentiation:[4]

Hemolytic (beta). Production of clear, well-developed zones of complete lysis of the red blood cells around and beneath the colonies

Green or Greening (alpha hemolytic) Production of green discoloration of the red blood cells in the vicinity of the colony There may be partial hemolysis in addition to the green discoloration, especially after refrigeration.

Indifferent (gamma hemolytic). No effect on red blood cells

(i) *Beta Hemolytic Streptococci.* Biochemical tests for species identification are rarely required, but these streptococci should be classified serologically. Lancefield[5] has shown that the beta hemolytic streptococci can be divided into 'Groups' on the basis of a group-specific polysaccharide 'C' substance, identified by a precipitin reaction between the antigenic cellular extract and a homologous immune serum. Up to the present, 12 groups have been recognized, A, B, C, D, E, F, G, H, K, L, M, and N. About 95 per cent of the strains cultured from human infections belong to Group A However strains of Groups B, C, D, F, G, and

K have been isolated from severe, and at times fatal, infections. Groups G and K are not uncommon in throat cultures.

PREPARATION OF ANTIGEN. The formamide method [6] of preparing the antigen is the most practical. Use 18- to 24-hour cultures of the test strain in 1 per cent dextrose infusion broth. Centrifuge the culture for 15 min. Draw off the supernatant as completely as possible and discard. Add 2 cc. of formamide (use commercial preparation as it is) to the sediment and shake well. Heat at 150° to 180° C. for 15 min. in an oil bath. Cool and add 5 cc. acid alcohol (95 cc. of absolute ethyl alcohol plus 5 cc. of 2 N hydrochloric acid). Centrifuge the mixture for about 5 min. and pipette the supernatant into a small tube. Discard the precipitate. Add 1 cc. of acetone to the supernatant fluid and shake the tube. Then centrifuge the mixture for 1 min. and discard the supernatant. The precipitate is very small in amount but it contains practically all of the group antigen. Add 2 cc. of saline to the sediment and shake well. Then centrifuge the tube for about 5 min. and transfer the supernatant to a small tube. Add 2 to 3 drops of phenol-red indicator, and neutralize the whole with 1 per cent sodium carbonate (about 3 drops is required)

TECHNIC OF TEST. Pipette 0.1 cc. of each of the undiluted group sera into tubes, 6 by 50 mm. Add, without mixing, an equal amount of the extract to be tested. The formation of a ring at the zone of contact indicates a positive reaction. This ring may form almost immediately or may require 10 to 15 min. If no ring is formed with any of the group sera tested, shake the tubes to mix the serum and the extract and incubate at 37° C. for 2 hr. Examine for formation of a precipitate and if none is present, place the tubes in the refrigerator overnight, before the final reading is made. If no 'grouping' is obtainable, prepare a second extract and test, before classifying the strain as ungroupable.

Antisera for Groups A, B, and C may be purchased from firms specializing in biologic products. Antisera for the remaining Groups should be obtained by immunizing rabbits. (See *Diagnostic Procedures and Reagents*, American Public Health Association, 1st ed., pp. 194-5, New York, 1941.)

(ii) *Green and Indifferent Streptococci.* Green streptococci (alpha hemolytic) should be differentiated from pneumococci. Further differentiation of the green and indifferent streptococci may not be required. If species differentiation seems to be indicated, the reader is referred to Sherman's 'Review of the Streptococci' in *Bacteriological Reviews* 1-2: 3-95 (1938). The following key adapted from Jordan and Burrows [7] will prove helpful:

I. Viridans Group: Normal inhabitants of the mouth and nasal passages
 a. Ferments lactose
  (1) Starch hydrolyzed, does not survive 60° C. for 30 minutes, growth inhibited by bile.
   Strep. salivarius.

(2) Starch not hydrolyzed, survives 60° C for 30 minutes, bile tolerant.

Strep bovis.

b. Does not ferment lactose.

Strep equinus.

II. Lactic Group: Found in milk and milk products.

a. Ferments maltose and dextrin

Strep lactis

b. Does not ferment maltose or dextrin.

Strep. cremoris

III. Enterococcus Group. Normal inhabitant of the intestinal canal.

a. Does not liquefy gelatin

(1) Ferments mannitol and sorbitol

Strep faecalis

(Lancefield Group D)

b. Liquefies gelatin

(1) Variable alpha hemolysis.

Strep. liquefaciens.

In certain instances where these streptococci seem to be the chief or sole etiological agent in the infection, it may be desirable to group them with the immune sera used for beta hemolytic streptococci. Certain of the streptococci that do not produce hemolysin have serological properties apparently identical with the beta hemolytic streptococci[5] This fact may be of interest in evaluating the response of such infections to sulfonamide or penicillin therapy.

(b) *Pneumococci.* The pneumococci should be differentiated from the green streptococci by the bile solubility test.

RAPID PLATE TEST. Sprinkle the bile salt, sodium desoxycholate, over the suspected pneumococcal colonies on the blood-agar plate. Incubate the plate at 37° C for ½ hr. If the colonies are pneumococcal, they will have lysed

BROTH TEST. To 4 parts of a culture of the organisms in 0 2 per cent dextrose cooked heart medium add 1 part of a 10 per cent solution of sodium desoxycholate or sterile ox bile Incubate at 37° C. for 1 hr. If the organism is a pneumococcus, the cells will have lysed and the suspension cleared.

(i) *Typing.* The microserological test for capsular swelling in the presence of a specific antiserum is the most important method used in the identification of pneumococci. Since the advent of sulfonamide and penicillin therapy, rapid typing is of less importance. It may not be required. The 'typing' of pneumococci is facilitated by the use of the 6 polyvalent or pooled antisera, each pool representing a combination of 3 to 6 type-specific sera. When the 'Quelling' reaction occurs with one of these pools, the material is then tested with each of the univalent antisera contained in the pool.

If the Gram-stained preparations of specimens such as sputum, pleural, spinal, or other body fluids show fairly numerous lanceolate diplococci, the material may be typed directly. If only a few or no diplococci are seen in the preparation, and a rapid test is desirable, the sputum or other material should be centrifuged and washed. A saline suspension of the sediment should be injected intraperitoneally into a mouse. As soon as there is evidence of an accumulation of fluid in the abdomen (4 to 8 hours), the fluid should be withdrawn with a capillary pipette and typed A Gram-stained preparation should also be made to establish the presence or absence of lanceolate diplococci. If the mouse dies, the peritoneal fluid and heart's blood should be cultured, and saline washings of the peritoneal cavity typed.

All cultures sent to the laboratory should be planted in 0 2 per cent dextrose meat medium and on blood-agar plates regardless of the results of any direct serological tests. A slide test for 'Quelling' may be made with the meat media cultures or with colonies fished from blood-agar plates.

TECHNIC OF TEST. On a glass slide mix with the undiluted antisera the material to be typed. The volume of the serum should be from 3 to 6 times that of the specimen. Add a large loopful of Loeffler's alkaline methylene blue, mix the preparation thoroughly and apply a cover slip. Allow the mixture to stand for a few minutes and then examine under an oil-immersion objective In the presence of homologous antiserum, the swelling of the capsule usually occurs promptly, but the reaction may take as long as 30 min. The bacterial bodies are stained blue, while the swollen capsule is opalescent. The test is first performed with the polyvalent sera, and then with each of the univalent sera in the pool with which it reacts.

Certain pneumococci will not type, and the bile-solubility test must be relied upon to establish identification.

(c) *Staphylococci and Related Micrococci.* In diagnostic work in view of the tentative nature of all classification schemes, we believe that the emphasis should be placed on the production of coagulase for the differentiation of potentially pathogenic from nonpathogenic strains. If 'species' differentiation is required, the following key, which represents a condensation by Dr. I. C. Hall [8] of Hucker's classification, is useful:

Colonies on plain agar-yellow	
Gelatin liquefied	
Nitrates reduced	*M. citreus (Staphylococcus citreus)*
Nitrates not reduced	*M. flavus*
Gelatin not liquefied	
Nitrates reduced	*M. varians*
Nitrates not reduced	*M. luteus*

Colonies on plain agar-golden or buff	
Gelatin liquefied	*M aureus (Staphylococcus aureus)*
Gelatin not liquefied	*M aurantiacus*
Colonies on plain agar-white	
Gelatin liquefied	
Nitrates reduced	*M albus (Staphylococcus albus)*
Nitrates not reduced	*M ureae*
Gelatin not liquefied	
Nitrates reduced	*M epidermidis*
Nitrates not reduced	*M. candidus*

The great majority of *Staphylococcus aureus* strains are coagulase positive, strains of *Staphylococcus albus* or *Micrococcus aurantiacus* may be coagulase-positive, but the other species rarely, if ever, produce coagulase.

LIQUEFACTION OF GELATIN TEST. Inoculate Medium #13 with 0 1 cc of an overnight culture of the test strain in beef-infusion broth Incubate the tubes at 37° for 10 days and then test for the liquefaction of gelatin by immersion in ice water for 30 min. This period of incubation represents a compromise but a longer period is not practical in diagnostic laboratories.

NITRATE REDUCTION TEST To 5 cc. of nitrate broth (Medium #9) add 0.25 cc of an overnight culture of the test strain. Incubate for 24 hr. at 37°. Add 1 cc. of nitrate solution #1, shake the tubes, and add 1 cc. of nitrate solution #2 The development of a red color indicates the reduction of nitrates to nitrites. For formula of test reagents see under 'Clostridia'

(i) *Special Differentiation of Staphylococci (Micrococci).* In addition to differentiation on the basis of hemolytic activity and pigment production, the staphylococci must be tested for the production of coagulase, that is, their ability to clot plasma, as this is the best single criterion for the differentiation of pathogenic from nonpathogenic staphylococci Tests for the fermentation of mannitol are optional, although a positive result is important confirmatory evidence of potential pathogenicity.

COAGULASE PRODUCTION To 1 cc. of a 6-to-24-hr. broth culture of staphylococcus or to 1 cc. of a saline suspension of staphylococcal colonies add 1 cc of citrated human plasma (human is best, but rabbit or horse plasma may be used). Incubate the mixture at 37° C and after 4 to 6 hr. make readings The majority of coagulase positive strains clot the plasma within 4 hours, occasionally a strain takes longer. Any degree of clotting constitutes a positive reaction.

If colonies of staphylococci are picked from a plate and transferred directly to a drop of fresh human plasma on a glass slide, coagulase positive strains clump almost immediately. This is a simple and rapid method for differentiating pathogenic from nonpathogenic strains.[9-10]

B. anthracis	*B. subtilis* group
Nonmotile	Motile
Encapsulated	Nonencapsulated
Gelatin slowly liquefied	Gelatin rapidly liquefied
Grows in long chains	Grows in short chains
No turbidity in broth	Turbidity in broth
Inverted fir-tree growth in gelatin	Fir-tree growth absent or atypical
Pathogenic to laboratory animals	Nonpathogenic to laboratory animals

If there is reason to suspect anthrax infection, the animal pathogenicity tests must be carried out.

TEST.[14] A white mouse or a guinea pig is injected subcutaneously with 0.5 cc. of a 24-hr. culture in 0.2 per cent dextrose cooked meat medium. The animal usually dies within 12 to 48 hr. At autopsy, there is a hemorrhagic gelatinous exudate at the site of inoculation. The bacilli may be seen in stained preparations of the blood, liver, and spleen. Cultures should be made of these tissues and examined for characteristic cell and colony morphology.

(ii) *Bacillus subtilis group.* No further differentiation is necessary. The species commonly found are *B. subtilis, B. mesentericus, B. mycoides,* and *B. vulgatus.*

(b) *Nonsporulating Bacilli.*

(i) *Mycobacterium tuberculosis.*

Special media for cultivation of tubercle bacilli. The demonstration of acid-fast bacilli in a Ziehl-Nielson stained preparation is not sufficient evidence for a laboratory diagnosis. The typical pathological lesion must be produced in a guinea pig. The specimen may also be planted on Petroff's (Medium #24) or Petragnani's medium (Medium #25) for *in vitro* cultivation. If bacterial forms other than acid-fast bacilli are present in the stained preparation, the culture material must be digested before animal pathogenicity tests are made or before media are inoculated.

In vitro cultivation: Colonies of the tubercle bacillus appearing on the surface of the media have a typical bread-crumb appearance. At first they are whitish yellow and moist but turn brownish and become dry as the culture ages. Since all cultures must be incubated for 8 weeks before being discarded as negative, care must be taken to avoid drying of the medium. A rubber cap should be placed over the cotton stopper. For further details of cultural methods, the reader is referred to the work of MacNabb[15] and Dubos.[16]

SEPARATION OF MIXED CULTURES BY DIGESTION. The material to be digested is put into a test tube and an equal volume of 3 per cent hydrochloric acid is added. The acid should contain a bromcresol purple indicator. The tube is stoppered and the contents well mixed. Care must be taken to avoid wetting the cotton plug. After the mixture has stood at room temperature for 2 hours,

it is made slightly alkaline to the bromcresol purple indicator with 3 per cent sodium hydroxide. It is then centrifuged, and the supernatant decanted. The sediment is used for pathogenicity tests and for the inoculation of culture media

It may be necessary to concentrate the material before digestion. A summary of the methods used will be found in *Laboratory Methods of the United States Army* (published by Lea and Febiger, 1944), page 501.

ANIMAL PATHOGENICITY TESTS. A guinea pig is injected in the groin with a small amount of the material as received or the digested sediment suspended in saline. The pig is examined weekly and as soon as there is evidence of a lesion, it is killed and autopsied If macroscopic lesions are present in the glands, liver, spleen, or lungs, a Ziehl-Nielson stained preparation is examined for the presence of acid-fast bacilli, and specimens should be sent to the pathological laboratory for sectioning. If the animal dies, the liver, spleen, and lungs should be sectioned even if no macroscopic lesions are seen Animals that are still alive after 10 weeks should be killed and autopsied. If there is suspicious gross pathology, the tissues should be sent to the pathological laboratory.

(ii) *Corynebacterium*. At times, it may be necessary to differentiate between the diphtheria bacillus and diphtheroid bacilli. The diphtheria bacillus is occasionally found in surgical infections, particularly in chronic ulcers or slow-healing wounds. If a diphtheroid-like bacillus seems to be the sole etiological agent in an infection, the carbohydrate fermentation tests should be made. If cultures are received from the mouth in children, they should be plated on Loeffler's serum medium (Medium #19) and Tellurite medium (Medium #22) to rule out the presence of the diphtheria bacillus. Culturally pure strains should be inoculated into carbohydrate serum water (Medium #11a).

TABLE II

FERMENTATION REACTIONS OF CORYNEBACTERIA

ORGANISMS	PRODUCTION OF ACID IN MEDIA CONTAINING		
	Dextrose	Maltose	Saccharose
C diphtheriae	Acid	Acid	No acid
C. hofmanni	No acid	No acid	No acid
C. xerosis	Acid	Acid	Acid

Readings are made after 24 and 48 hours' incubation at 37° C. The identity and virulence of the diphtheria bacillus can only be finally established by animal inoculation. For a further discussion of the morphologic and biochemical characteristics of the diphtheria bacillus, see Frobisher.[17]

(2) *Gram-Negative Bacilli:*

(a) *Sporulating.* These need not be further differentiated.

(b) *Nonsporulating Bacilli.*

(i) *Test Media.* Culturally pure strains should be inoculated from the blood-agar plate (Medium #15a) into:

1. Plain agar plates (Medium #15) for pigment formation
2. Gelatin stabs (Medium #13) for gelatin liquefaction
3. Plain Bacto-peptone water (Medium #7) for indol production
4. Dextrose Bacto-peptone water (Medium #7a) for dextrose fermentation
5. Lactose Bacto-peptone water (Medium #7a) for lactose fermentation
6. Clark-Lubs Medium (Medium #8) for methyl red and Voges-Proskauer tests

(ii) *Scheme of Study.* The following chart is a rapid method of identification. Certain bacteria may fail to fall into any of the described groups and will then have to be studied individually. Intermediate forms between *Escherichia* and *Aerobacter* and late lactose-fermenting bacilli may cause confusion. Reference to Bergey's *Manual*[12] or Jordan and Burrows[18] will provide final identification.

(a) Bacteria failing to grow in absence of hemoglobin: *Hemophileae*

(b) Bacteria producing a green, blue or yellow-green pigment which diffuses through medium (Particularly gelatin or plain agar). *Pseudomonas*

(c) Bacteria liquefying gelatin, fermenting dextrose with acid and gas, and having spreading colonies: *Proteus*

(d) Bacteria which do not fall into groups (a), (b) or (c):
- (d-1) Not fermenting dextrose or lactose
  - *Stain for morphology*
    - (1) Tiny bacilli: *Brucella*
    - (2) Coliform bacilli: *Alkaligenes*
- (d-2) Fermenting dextrose and lactose with acid and gas formation.
  - Stain for morphology
    - (d-2a) Encapsulated bacilli: *Klebsiella*
      These should be typed with Friedlander A and B antisera.

(d-2b) Indol positive, methyl red positive, Voges-Proskauer negative: *Escherichia*

(d-2c) Indol negative, methyl red negative, Voges-Proskauer positive: *Aerobacter*

(d-2d) Atypical reactions: (1) Coli intermediates, (2) *Coli mutabile*, (3) paracolon bacillus.

(d-3) Fermenting dextrose with acid and gas but failing to ferment lactose.

(d-3a) Indol negative, Voges-Proskauer negative, motile: *Salmonella*

(d-4) Fermenting dextrose with acid but without gas, failing to ferment lactose:

Stain for morphology

(d-4a) Tiny bacilli with bipolar stainings· *Pasturella*

(d-4b) Larger bacilli without bipolar stainings.

(d-4b-1) Motile—*Eberthella*

(d-4b-2) Nonmotile—*Shigella*

Strains falling into the *Salmonella, Eberthella,* or *Shigella* groups should be tested for agglutination with specific antisera wherever possible to establish final identification.

(iii) *Details of Special Tests for Aerobic Gram-Negative Nonsporulating Bacilli*

GELATIN STABS. Incubate cultures for 5 days at 37° C., then place in refrigerator for 2 hours to determine whether or not actual liquefaction has occurred.

CARBOHYDRATE FERMENTATIONS Read for acid, acid and gas, no acid and no gas Do not make final readings on fermentations earlier than 72 hr. Late lactose-fermenting strains may not show fermentation until after 7 to 15 days' incubation. Gas production is observed in the small inverted tube submerged in the medium.

INDOL TEST To a 48-hr. peptone-water culture (Medium #7) add 1 cc. of ether to 5 cc. of culture and shake well Allow 0 5 cc. to 1 cc of Ehrlich's reagent to run down side of tube and layer between culture and ether. Test is positive when a pink to rose color forms at juncture of reagents.

Ehrlich's Reagent:

Paradimethylaminobenzaldehyde	4 gm.
95 per cent ethyl alcohol	380 cc.
Conc. hydrochloric acid	88 cc.

METHYL-RED TEST. To 1 cc. of a 96-hr. culture in Clark-Lubs medium (Medium #8) add 1 cc. of methyl-red indicator. Bright red color indicates a positive test, yellow color a negative test.

VOGES-PROSKAUER TEST. To 1 cc of a 96-hr. culture in Clark-Lubs medium (Medium #8) add 0 6 cc. of 5 per cent alpha naphthol in 95 per cent ethyl

alcohol. Shake well. Add 0.2 cc. of 40 per cent potassium hydroxide and shake. A positive reaction is indicated by the development of a pink color in the upper fluid of the medium in 2 to 5 min. This color deepens in 30 to 60 min. to crimson or magenta.

MOTILITY TEST. Determine motility by examining under a microscope a hanging drop preparation of a 6-hr. culture in broth. At times it is desirable to study the motility of bacteria from colonies on solid media. A suspension of the colony may be made in a drop of saline, and the hanging drop preparation examined microscopically.

The drop is placed on a glass cover slip which is then inverted over a cup-shaped depression in a special glass slide. The depression is first lined with vaseline around the edge to prevent evaporation and the formation of convection currents. A distinction must be made between 'Brownian' movement and true motility. The bacilli should move across the field for a distance of at least 10 to 20 times their length.

2. *Anaerobic and Microaerophilic Bacteria*

A. ANAEROBIC COCCI

(1) Gram-Positive Cocci:

(a) *Streptococci.* No streptococcus, either hemolytic or nonhemolytic, should be classified as an obligate anaerobe unless after 10 generations on laboratory media it still fails to grow on the surface of blood-agar plates incubated aerobically.

(i) Anaerobic Hemolytic Streptococci. Obligate anaerobic hemolytic streptococci are rare. As a rule, the hemolytic streptococci that grow only on the anaerobic plate on primary isolation from infectious processes accustom themselves rapidly to atmospheric oxygen tension, and grow aerobically as well as anaerobically after 2 to 4 transplants on laboratory media. Such strains should be reported as 'microaerophilic.' When the spreading of an equal amount of inoculum on the surface of two blood-agar plates, one of which is incubated aerobically, the other anaerobically, results in the development of a much greater number of colonies on the anaerobic plate, this strain should also be considered 'microaerophilic' since it is evident that only occasional individuals in the strain can multiply under aerobic conditions. Some strains show growth on the aerobic plate only after 48 to 72 hours' incubation, although typical well-developed colonies are present on the anaerobic plate after 24 hours' incubation. These strains should likewise be termed 'microaerophilic' as growth is definitely retarded under aerobic conditions. Such organisms are the agents of chronic undermining, burrowing ulcers.

The anaerobic and microaerophilic hemolytic streptococci should be

classified serologically (Lancefield Groups). The technic is the same as for the aerobic strains.

(ii) *Anaerobic Nonhemolytic Streptococci.* Obligate anaerobic nonhemolytic streptococci are not uncommon in surgical infections. Microaerophilic nonhemolytic streptococci are also frequently found. Although the microaerophilic nonhemolytic streptococci do not accustom themselves to atmospheric oxygen tension as readily as do the microaerophilic hemolytic streptococci, any nonhemolytic streptococcus that fails to grow on the surface of a blood-agar plate incubated aerobically after 10 transplants on laboratory media may be safely assumed to be an obligate anaerobe.

No adequate scheme for the classification of the anaerobic nonhemolytic streptococci is available. They may be roughly grouped as (1) producing gas and fetid odor, and (2) producing no gas and no odor. Prévot[19] lists a number of species with differential biologic characteristics. Many of the strains isolated in our laboratory failed to fall into any of these species. Colebrook and Hare[20] have attempted a classification on the basis of colony morphology, but this also has certain pitfalls.

If an attempt at species identification of the anaerobic nonhemolytic streptococci seems desirable, the following key taken from Bergey's *Manual of Determinative Bacteriology*[12] may be used:

DIFFERENTIATION OF SPECIES OF ANAEROBIC NONHEMOLYTIC STREPTOCOCCI

(A) *Gas and Fetid Odor Produced*
1. No general turbidity in broth
   a Acid from maltose
      Streptococcus anaerobius
   b. No acid from maltose
      Streptococcus foetidus
2. Turbidity in broth
   a. No gas in semisolid agar (Veillon). No gas in peptone water
      Streptococcus putridus
   b. Abundant gas in semisolid agar (Veillon). Gas in peptone water
      Streptococcus lanceolatus

(B) *No Gas and No Fetid Odor Produced*
1 Milk not coagulated
      Streptococcus micros
2. Milk coagulated
   a Viscous sediment in broth Semisolid agar colonies blacken with age
      Streptococcus parvulus
   b. No viscous sediment in broth Semisolid agar colonies do not blacken with age
      Streptococcus intermedius

For morphological and additional cultural characteristics, the reader is referred to *Les Microbes anaerobies* by Weinberg, Nativelle, and Prévot (Masson et Cie., Paris, 1937), pages 989-1018.

The microaerophilic nonhemolytic streptococci need not be further differentiated. Prévot[19] lists one species 'evolutus' characterized by the liquefaction of gelatin, but not all strains have this property. This is the essential organism in the advancing periphery of the lesion described in Chapter XIV as 'Progressive Bacterial Synergistic Gangrene.'

(b) *Staphylococci.* The anaerobic staphylococci may be differentiated as follows:

(i) No gas produced in culture: *Staphylococcus anaerobius* (common)
(ii) Gas produced in culture
  a. Acid from dextrose: *Staphylococcus aerogenes* (common)
  b. No acid from dextrose: *Staphylococcus asaccharolyticus* (rare)

(c) *Other Anaerobic Gram-Positive Cocci.* Anaerobic and microaerophilic pneumococci should be typed. The technic is the same as for aerobic strains. Sarcina and Gaffkia need be differentiated on the basis of morphology only.

(2) *Gram-Negative Cocci:* These should be differentiated on the basis of cell morphology in liquid media.

(a) *Anaerobic Neisseria.* These usually grow in pairs with adjacent sides flattened, and the individual cocci are approximately 0.6 μ in diameter. If further differentiation of the Neisseria is required, see Bergey's *Manual of Determinative Bacteriology.*[12]

(b) *Veillonella.* These grow in irregular masses and the individual cocci are very small, about 0.3 μ in diameter. The two species of *Veillonella* may be differentiated by biochemical reactions in semisolid agar as shown in the accompanying table. (For the indol test use Medium #30, for nitrate reduction, Medium #28, for fermentation of dextrose, Medium #30a. For technic of tests, see under Clostridia, pp. 311-12.)

TABLE III

DIFFERENTIATION OF VEILLONELLA

ORGANISM	INDOL PRODUCTION	NITRATES REDUCED TO NITRITES	FERMENTATION OF DEXTROSE
V. parvula	+	+	+
V. gasogenes	−	−	−

B. ANAEROBIC GRAM-POSITIVE BACILLI

(1) *Sporulating Bacilli (Clostridia):* The classification of these bacilli must be carried through to species identification. The methods outlined by Hall,[21] Spray,[22] and Reed and Orr[23] have proved to be of great value in rapid identification. These entail a study of (a) the motility, (b) the morphology of the bacillus including the position and form of the spore, (c) the reaction in iron milk, (d) the liquefaction of gelatin, (e) the fermentation of carbohydrates, (f) the reduction of nitrates to nitrites, (g) the production of indol, (h) the production of hydrogen sulfide, and (i) the colony morphology on blood-agar plates. The final identification of the pathogenic clostridia rests on protection of the laboratory animal with a specific antiserum.

(a) *Technic of Tests.* Use young cultures, not over 24 hours' old, in plain meat medium (Medium #3) to seed the differential media. Boil all diagnostic media for 5 min., cool rapidly to 40-45° C. and inoculate immediately with the test strain. Deliver the inoculum into a semisolid medium to the bottom of the tube. It is not necessary to incubate the semisolid medium cultures in an anaerobic jar.

(i) *Motility.* Tests should be made with young cultures. As a routine procedure, we fish colonies from the anaerobic blood-agar plate after 24 hours' incubation, emulsify them in a drop of saline on a glass cover slip, invert over a hollow ground slide, and examine immediately under an oil-immersion objective. If motility is not observed, repeat the test, using a 0.2 per cent dextrose cooked heart medium culture as soon as there is visible turbidity. With some species, such as *C. bifermentans,* several preparations may have to be examined before true motility is seen.

(ii) *Morphology* The morphology is studied in a Gram-stained preparation on a glass slide. Unusual care is required to avoid overdecolorizing the bacilli. All species are Gram-positive in young cultures, but in older cultures some may be Gram-negative. When the spore has matured, the body of the bacillus is often Gram-negative.

SPORES. The position and shape of the spore is noted. The spore positions are designated as central, excentric, subterminal and terminal, the shape as oval or spherical Some spores distort or 'swell' the rod, others do not. Observations on the relative abundance of the spores should be made. Some species, such as *C. sporogenes, C. sordellii,* and *C. bifermentans,* sporulate readily and abundantly. A smear of a 48-hour-old colony on a blood-agar plate may show a preponderance of free spore forms. In others, such as *C. paraputrificum* and *C. sphenoides,* sporulating bacteria are rare. When spore formation in the cooked heart medium or in surface colonies on anaerobic blood-agar plates is absent or inadequate for detailed observations of characteristic morphology,

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V. gasogenes	−	−	−

B. ANAEROBIC GRAM-POSITIVE BACILLI

(1) *Sporulating Bacilli (Clostridia)*: The classification of these bacilli must be carried through to species identification. The methods outlined by Hall,[21] Spray,[22] and Reed and Orr[23] have proved to be of great value in rapid identification. These entail a study of (a) the motility, (b) the morphology of the bacillus including the position and form of the spore, (c) the reaction in iron milk, (d) the liquefaction of gelatin, (e) the fermentation of carbohydrates, (f) the reduction of nitrates to nitrites, (g) the production of indol, (h) the production of hydrogen sulfide, and (i) the colony morphology on blood-agar plates. The final identification of the pathogenic clostridia rests on protection of the laboratory animal with a specific antiserum.

(a) *Technic of Tests.* Use young cultures, not over 24 hours' old, in plain meat medium (Medium #3) to seed the differential media. Boil all diagnostic media for 5 min, cool rapidly to 40-45° C. and inoculate immediately with the test strain. Deliver the inoculum into a semisolid medium to the bottom of the tube. It is not necessary to incubate the semisolid medium cultures in an anaerobic jar.

(i) *Motility.* Tests should be made with young cultures. As a routine procedure, we fish colonies from the anaerobic blood-agar plate after 24 hours' incubation, emulsify them in a drop of saline on a glass cover slip, invert over a hollow ground slide, and examine immediately under an oil-immersion objective. If motility is not observed, repeat the test, using a 0.2 per cent dextrose cooked heart medium culture as soon as there is visible turbidity. With some species, such as *C. bifermentans*, several preparations may have to be examined before true motility is seen.

(ii) *Morphology.* The morphology is studied in a Gram-stained preparation on a glass slide. Unusual care is required to avoid overdecolorizing the bacilli. All species are Gram-positive in young cultures, but in older cultures some may be Gram-negative. When the spore has matured, the body of the bacillus is often Gram-negative.

SPORES The position and shape of the spore is noted. The spore positions are designated as central, excentric, subterminal and terminal, the shape as oval or spherical Some spores distort or 'swell' the rod, others do not. Observations on the relative abundance of the spores should be made. Some species, such as *C. sporogenes*, *C. sordellii*, and *C. bifermentans*, sporulate readily and abundantly. A smear of a 48-hour-old colony on a blood-agar plate may show a preponderance of free spore forms In others, such as *C. paraputrificum* and *C sphenoides*, sporulating bacteria are rare. When spore formation in the cooked heart medium or in surface colonies on anaerobic blood-agar plates is absent or inadequate for detailed observations of characteristic morphology,

For morphological and additional cultural characteristics, the reader is referred to *Les Microbes anaerobies* by Weinberg, Nativelle, and Prévot (Masson et Cie., Paris, 1937), pages 989-1018

The microaerophilic nonhemolytic streptococci need not be further differentiated. Prévot [19] lists one species 'evolutus' characterized by the liquefaction of gelatin, but not all strains have this property. This is the essential organism in the advancing periphery of the lesion described in Chapter XIV as 'Progressive Bacterial Synergistic Gangrene.'

(b) *Staphylococci.* The anaerobic staphylococci may be differentiated as follows:

(i) No gas produced in culture: *Staphylococcus anaerobius* (common)

(ii) Gas produced in culture

a. Acid from dextrose: *Staphylococcus aerogenes* (common)

b. No acid from dextrose: *Staphylococcus asaccharolyticus* (rare)

(c) *Other Anaerobic Gram-Positive Cocci.* Anaerobic and microaerophilic pneumococci should be typed. The technic is the same as for aerobic strains. Sarcina and Gaffkia need be differentiated on the basis of morphology only.

(2) *Gram-Negative Cocci:* These should be differentiated on the basis of cell morphology in liquid media.

(a) *Anaerobic Neisseria.* These usually grow in pairs with adjacent sides flattened, and the individual cocci are approximately 0.6 μ in diameter. If further differentiation of the Neisseria is required, see Bergey's *Manual of Determinative Bacteriology.*[12]

(b) *Veillonella.* These grow in irregular masses and the individual cocci are very small, about 0.3 μ in diameter. The two species of *Veillonella* may be differentiated by biochemical reactions in semisolid aga as shown in the accompanying table. (For the indol test use Medium #30, for nitrate reduction, Medium #28, for fermentation of dextrose, Medium #30a. For technic of tests, see under Clostridia, pp. 311-12)

TABLE III
DIFFERENTIATION OF VEILLONELLA

ORGANISM	INDOL PRODUCTION	NITRATES REDUCED TO NITRITES	FERMENTATION OF DEXTROSE
V. parvula	+	+	+
V. gasogenes	–	–	–

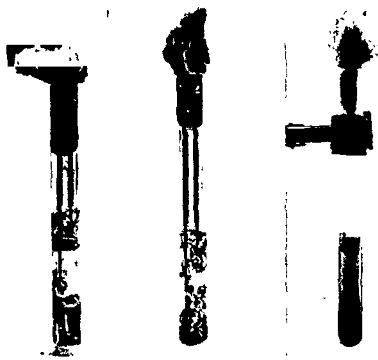


FIG. 3. (a) The 'stormy' fermentation of milk by *C. welchii*. The milk is clotted by the formation of acid and the gas then breaks up the clot. (b) The blackening of iron milk by putrefactive anaerobes.

days. This is a valuable presumptive test for *C. histolyticum*. We have encountered strains of *C. welchii* that failed to liquefy gelatin, but they are rare.

(v) *Carbohydrate Fermentations.* Medium #30a is used for these tests. Only dextrose, lactose, saccharose, and salicin need be used for the identification of the great majority of the clostridia. It is not practical to include the Andrade indicator in the medium since certain metabolic products of the bacilli destroy it. A portion of the medium may be withdrawn after 36 hours' incubation and tested for acidity. If no acid has been formed, the tests should be repeated up to 7 days. Fermentation is evidenced by a definite acid reaction when contrasted to the sugar-free control.

(vi) *Reduction of Nitrates to Nitrites.* The seeded medium (Medium #28) is usually incubated for 72 hours before the test is made, although reduction often occurs within 24 hours.

TECHNIC OF TEST. 1 cc. of nitrate test reagent #1 is added to about 5 cc. of the culture in the nitrate semisolid agar. The contents of the tube are well

the following technic, which was suggested to us by Dr. I. C. Hall,[8] may be used. The culture is inoculated on the surface of a blood-agar slant which is then incubated in an anaerobic jar for 48 to 72 hours. The slant is then immersed in boiling water for 1 minute, cooled, and reincubated for 48 hours. Subcultures are made into 0.2 per cent dextrose cooked meat medium. In this way, the individual bacilli that do not form spores readily are eliminated, and the culture represents the growth of the more active spore-formers only. If the sporulation is still inadequate, this procedure may be repeated several times.

(iii) *Reaction in Iron Milk.* We have found the distinctive reactions in iron milk (Medium #12) of the greatest importance. Spray[22] in his key makes the primary divisions for the identification of the clostridia on this basis.

'STORMY' FERMENTATION. The milk first clots and then the clot is riddled with gas bubbles and blown to bits. The clot is not digested or blackened. Such a fermentation is presumptive evidence of *C. welchii* if it occurs within 6 to 18 hours, since this reaction is delayed (48-72 hours) in the other species that produce it (*C. butyricum* and *C. multifermentans*). Some workers consider that when this stormy fermentation is produced by a large, thick, Gram-positive, nonmotile bacillus, no further identification tests for *C. welchii* are necessary (Fig. 3a).

DIGESTION AND BLACKENING OF IRON MILK. The speed of the reaction in this group differs. Within 12 to 24 hours, the milk becomes watery and has a yellowish translucence. A coarse flocculation then develops. With *C. sporogenes* this may become coal black within 24 hours. With *C. sordellii*, *C. bifermentans*, and *C. histolyticum* the intense blackening may not appear until after 48 to 72 hours of incubation (Fig. 3b).

INACTIVE GASEOUS FERMENTATION, LATE, IF ANY, COAGULATION, OF IRON MILK. Spray divides the species that produce 'inactive gaseous fermentation' with no digestion of the clot and no blackening of the iron milk into two groups on the basis of whether there is delayed clot formation (4-6 days), for example, *C. septicum*, or infrequent coagulation, which rarely occurs before 12 days, for example, *C. novyi*. For rapid identification of organisms this division into two groups is of doubtful value.

NO CHANGE IN IRON MILK. This group includes *C. tetani*.

(iv) *Liquefaction of Gelatin.* Iron gelatin (Medium #14) is used for this test. The cultures are incubated for 24 hours and then tested by immersion in ice water. If no liquefaction is evident the cultures are incubated further and tested daily. In the absence of liquefaction, if the identity of the bacillus is in doubt, the incubation should be continued for at least 30 days. In iron gelatin *C. histolyticum* produces a distinctive orange to wine-red color.[22] Fading may occur after 3 to 5

genes. *Clostridium sordellii* forms a stellate colony with its long axis along the streak of the nichrome needle or spatula.

The colonies of bacilli that sporulate abundantly are usually opaque in consistency after 48 hours' incubation. In smears made from such opaque colonies, relatively few vegetative forms are seen, but free spores are abundant. This is particularly striking with *C. sordellii* and *C. bifermentans*. After 48 hours' incubation, the colonies of *C. sporogenes* often resemble a piece of raveled wool. They are whitish yellow with a distinct fringed periphery.

Tetanus is characterized by a spreading growth that hemolyzes the red blood cells and may cover the entire plate with a fine film. However, the colonies of *C. septicum* and *C. novyi* also spread, especially on moist plates.

Description of colonial forms in reports by different workers vary. These are difficult to assay. The production of a zone of clear hemolysis or greening around the colony may depend upon the type of blood added to the agar, i.e. human, horse, rabbit, or sheep. The spreading proclivities of the colonies may depend upon the hardness of the agar used and upon the moisture content of the environment. Colonies on soft agar (1.5 per cent) are more likely to spread than those on 2 to 3 per cent agar. If the anaerobic jar is not evacuated before hydrogen is introduced or if a layer of anhydrous calcium chloride is not placed on the bottom of the jar, considerable water and water vapor may be present within the jar during the incubation period.

The clostridia may also show rough and smooth colonial variations. A great deal of care would have to be exercised before any clostridium was excluded from a species classification because of colonial characteristics

(x) *Animal Pathogenicity Tests.* It is our practice to establish the pathogenicity of *C. welchii* strains only when the organism has been isolated from an infection in which the bacillus appears to be playing an active part. *Clostridium sordellii* can only be distinguished from *C. bifermentans* by the animal pathogenicity test This organism is at times referred to as a toxic *C bifermentans*. Likewise, *C. botulinum*, types A and B, may be wrongly identified as *C. sporogenes* unless the pathogenicity of the culture is tested. We prefer to use guinea pigs, although mice and pigeons may be used. Pigeons do not develop clinical tetanus.

TEST. The guinea pig is inoculated intramuscularly in the groin with 0 25 cc. of an overnight culture of the test strain in 0.2 per cent dextrose cooked meat medium (Medium #3). The pathogenic species usually kill the pig within 24 hours with the production of the characteristic pathologic lesion (see

mixed and 1 cc. of nitrate test reagent #2 is then added. If the nitrate has been reduced to nitrite, a pink to cherry red color develops.

TEST REAGENTS.

Solution #1. Sulfanilic acid . . . . 0 5 gm
Acetic acid, sp. gr. 1.04 . . . . . 150 cc.

Solution #2. Boil 0.12 gm. of dimethylalphanaphthylamine in 20 cc. distilled water. Filter if necessary. Add 180 cc. acetic acid, sp. gr. 1.04.

Acetic acid, sp. gr. 1.04 is prepared by diluting 400 cc. conc. acid, sp. gr. 175 with 700 cc. distilled water.

(vii) *Production of Indol.* Medium #30 is used. The majority of strains that produce indol give a positive test after 24 hours' incubation A final test should be made after 72 hours' incubation. Ehrlich's reagent is used. See under tests for 'aerobic Gram-negative nonsporulating bacilli' (p. 305).

(viii) *Production of Hydrogen Sulfide.* Medium #29 is used. Spray[22] mentions three different types of reaction in the medium he advocates We have had considerable difficulty in the interpretation of the differential reactions he describes. The medium advocated by Reed and Orr[23] is more satisfactory, although these authors state that small traces of hydrogen sulfide produce no discoloration. When hydrogen sulfide is formed in moderate or large amounts, a definite blackening occurs. We have not found this to be an important key reaction in identifying the clostridia.

(ix) *Colony Formation on Anaerobic Blood-Agar Plates.* All of the clostridia discussed here are obligate anaerobes, i.e. will not grow on the surface of aerobic blood-agar plates, with the exception of *C. histolyticum* and *C. tertium.* These two species are microaerophiles. Surface growth under aerobic conditions is feeble, and spores are rarely if ever formed.

On blood-agar plates in the anaerobic jar, *C. welchii* produces a unique and characteristic zoning. The target appearance (inner zone of complete hemolysis, dark outer zone) with the colony forming the bull's eye is so characteristic that it constitutes presumptive evidence of hemolytic *C. welchii.* Some strains show only the outer zone, the clear inner zone being absent. These are the nonhemolytic strains. Both strains are pathogenic. The clear inner zone is produced by the theta hemolysin, which plays no essential part in the pathogenicity of the strain.[24]

Clostridium septicum produces a spreading hemolytic colony often a centimeter or more in diameter fusing with nearby colonies. *Clostridium novyi* forms a small irregular colony that turns sheep's blood slightly green. It cannot always be distinguished from *C. sordellii* or *C. sporo-*

genes. *Clostridium sordellii* forms a stellate colony with its long axis along the streak of the nichrome needle or spatula.

The colonies of bacilli that sporulate abundantly are usually opaque in consistency after 48 hours' incubation. In smears made from such opaque colonies, relatively few vegetative forms are seen, but free spores are abundant. This is particularly striking with *C. sordellii* and *C. bifermentans*. After 48 hours' incubation, the colonies of *C. sporogenes* often resemble a piece of raveled wool. They are whitish yellow with a distinct fringed periphery.

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Description of colonial forms in reports by different workers vary. These are difficult to assay. The production of a zone of clear hemolysis or greening around the colony may depend upon the type of blood added to the agar, i.e. human, horse, rabbit, or sheep. The spreading proclivities of the colonies may depend upon the hardness of the agar used and upon the moisture content of the environment. Colonies on soft agar (1 5 per cent) are more likely to spread than those on 2 to 3 per cent agar. If the anaerobic jar is not evacuated before hydrogen is introduced or if a layer of anhydrous calcium chloride is not placed on the bottom of the jar, considerable water and water vapor may be present within the jar during the incubation period.

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TEST. The guinea pig is inoculated intramuscularly in the groin with 0.25 cc. of an overnight culture of the test strain in 0 2 per cent dextrose cooked meat medium (Medium #3). The pathogenic species usually kill the pig within 24 hours with the production of the characteristic pathologic lesion (see

Chapter IX). All animals should be autopsied and direct smears and cultures made from the lesion and from the heart's blood. The final identification of the pathogenic organisms rests on the protection of the animal with specific antiserum. It is not necessary to sacrifice the animals in which no lesion develops. It has been our practice to use these for further tests after a lapse of one to two weeks or longer.

For the rapid identification of pathogenic species other than *C. welchii* neutralization of toxins by specific antitoxins is the method of choice.

(b) *Diagnostic Reactions in the Key Differential Media.* The chart on p. 315 will give the diagnostic reactions in the key media previously described. We have included the motility of the bacilli, the spore morphology, and the pathogenicity for animals, as these are important characteristics in species identification.

(c) *Additional Cultural Characteristics.* (i) *Cooked Heart Medium.* Following the suggestion of Dr. I. C. Hall,[8] we have been adding a piece of soft iron wire to the 0.2 per cent dextrose cooked meat medium used to isolate these bacilli in pure culture. This has a definite diagnostic value and, in addition, often discloses the presence of a contaminating organism.

The putrefactive species, such as *C. sordellii, C. bifermentans, C. sporogenes,* and *C. histolyticum,* blacken the meat within 48 hours in the presence of iron, as a result of the production of iron sulfide. This is accompanied by digestion of the meat particles and the production of a very foul odor.

The saccharolytic species will not blacken the meat within this period. *Clostridium welchii* produces a blackening as the culture ages, but *C. septicum* and *C. novyi* do not. There is a characteristic reddening of the meat particles in cultures of *C. welchii* and *C. septicum,* more pronounced in the case of *C. welchii. Clostridium novyi* bleaches the meat.

Clostridium tetani and *C. putrificum* may blacken the meat particles but only after prolonged incubation, whereas *C. tetanomorphum* does not produce this reaction.

(ii) *Tyrosin Crystals.* As cooked heart medium cultures of *C. histolyticum* age, there is a deposition of white tyrosin crystals in the liquefied meat. A variety of *C. sporogenes,* known as *C. tyrosinogenes,* also forms these crystals in the meat medium.

(iii) *Acrolein Formation.*[23] This is a valuable test for the rapid identification of *C. welchii.* Two per cent glycerol broth (Medium #10), tubed 'deep,' is heavily inoculated with the test strain. This tube is then immediately seeded with *B. prodigiosis* or some other quickly growing aerobic organism. A control culture of *B. prodigiosus* alone is made in the glycerol broth. Both cultures are incubated aerobically overnight. Micro-

Table IV

Differentiation of Various Species of Clostridia

Species	Motility	Spores	Milk	Liquefaction of gelatin	Dextrose	Lactose	Salicin	Saccharose	Indol	Nitrate reduction	Hydrogen sulfide	Animal pathogenicity
C welchii	−	Oval, central to excentric	S F.	+	+	+	−	+	−	+	+	+
C butyricum	+	Oval, excentric to subterminal, *swelling rod*	S F	+	+	+	+	+	−	+	+	−
C. multifermentans	+	Oval, central to excentric, swelling rod	S F.	−	+	+	+	+	−	+	−	−
C *botulinus* A, B	+	Oval, subterminal, swelling rod	D&B	+	+	−	+	−	−	−	+	+
C. sporogenes	+	Oval, subterminal, swelling rod	D&B	+	+	−	−	−	−	−	+	−
C sordellii	+	Oval, excentric	D&B	+	+	−	−	−	+	−	+	+
C bifermentans	+	Oval, excentric	D&B	+	+	−	−	−	+	−	+	−
C. capitovalis	+	Oval, terminal, swelling	D&B	+	+	−	−	−	+	−	+	−
C. subterminalis	+	Oval, subterminal	D&B	+	−	−	−	−	+	−	+	−
C nonfermentans	+	Oval, central to excentric	D&B	+	−	−	−	−	+	−	+	−
C histolyticum *	+	Oval, subterminal, swelling	D&B	+ †	−	−	−	−	−	−	+	+
C novyi	+	Oval, subterminal, *swelling*	Acid	+	+	−	−	−	−	−	+	+
C. septicum	+	Oval, excentric to subterminal	Acid	+	+	+	+	−	−	+	+	+
C. paraputrificum	+	*Oval, terminal, swelling*	Acid	−	+	+	+	+	−	−	±	−
C. sphenoides	+	Spherical subterminal becoming terminal, swelling	Acid	−	+	+	+	+	+	−	+	−
C *tertium* *	+	Oval, terminal, swelling	Acid	−	+	+	+	+	−	+	±	−
C tetani	+	Spherical, terminal, swelling	No change	±	−	−	−	−	+	−	±	+
C. putrificum	+	Spherical, terminal, swelling	No change	+	−	−	−	−	+	−	+	−
C tetanomorphum	+	Spherical to ovoid, terminal, swelling	No change	−	+	−	−	−	+	−	−	−

S F.—Stormy fermentation.
D&B—Digestion and blackening
Acid—Inactive gaseous fermentation, late if any coagulation, acid reaction in milk.
* Microaerophilic.
† Distinctive port wine color.

scopical examination after 18 hours' incubation will show that both organisms have multiplied. Platings on aerobic and anaerobic blood-agar plates show that the acrolein produced by *C. welchii* has killed all organisms present. The *B. prodigiosus* in the control glycerol broth grows on the aerobic blood plate.

(2) *Nonsporulating Bacilli:* The characteristic morphology in 0.2 per cent dextrose cooked meat medium should be noted. Differentiation should be made on the basis of production of gas and fetid odor and motility. No further identification is required. Prévot[26] lists 33 species, but few of these have been subjected to critical study. Eggerth,[27] and Eggerth and Gagnon[28] give detailed descriptions of anaerobic Gram-positive nonsporulating bacilli isolated from human feces.

C. ANAEROBIC GRAM-NEGATIVE BACILLI

(1) *Nonsporulating Bacilli:* Differentiation on the basis of morphology is all that is required, since no adequate classification of this large group of organisms is available. (See Prévot,[26] and Lewis and Rettger.[29]) For the time being, it seems best to adopt the convention of placing them in the following groups:

(a) *Fusobacterium.* The so-called fusiform bacilli may be defined as anaerobic Gram-negative bacilli usually with tapering ends, which stain with more or less distinct granules. In surgical infections, they are chiefly found in lesions of the jaw, mouth, in pleural exudates, and in symbiosis with spirochaetes.

(b) *Bacteroides.* This group is subdivided into (1) simple rods–type species, *B. fragilis;* and (2) pleomorphic rods–type species, *B funduliformis-necrophorus.* The simple rods may be motile or nonmotile, with or without capsules. Colonies of this group are not distinctive except for *B. melaninogenicum,* which forms a black pigment on blood agar. If there are numerous colonies present, all the blood may be removed from the agar and the plate resemble a plain agar plate. The black pigment develops slowly, and may not be evident unless the plates are incubated for 7 to 10 days. *Bacteroides melaninogenicum* has a tendency to form symbiotic colonies with the anaerobic nonhemolytic streptococci It may require several fishings and replatings before it is obtained in pure culture.

The pleomorphic rods are characterized by the presence of spheroid or swollen bodies. At times it is possible to see in a pure culture coccoid forms, simple rods, spheroid bodies, and long branching filaments.

The isolation of these bacilli is simplified by the use of Media #5 and #16, since the growth of Gram-positive species present in mixed cultures is inhibited by the crystal violet. We have also found that the

inclusion of tyrothricin 1:10,000 in dextrose cooked meat medium simplifies the isolation of these Gram-negative organisms in mixed cultures since the multiplication of the Gram-positive strains is either completely or partially inhibited.

3. *Spirochetes*

A. TREPONEMA

(1) *Treponema pallidum*· No further identification other than the demonstration of motile spirochetes in fresh specimens in dark-field microscopic examination is required.

(2) *Treponema Associated with Fusospirochetal Diseases:* Demonstration of motile spirochetes in a dark-field preparation is usually sufficient for identification (see Chapter IX). Spirochetes multiply in Hiss serum water (Medium #11) in the presence of the other organisms associated with fusospirochetal infections If isolation in pure culture is desirable, the technic developed by Rosebury and Foley should be used.[30]

4. *Fungi*

A. ACTINOMYCETES

These organisms together with the Mycobacteria and the Corynebacteria are considered to belong to the 'Higher Bacteria,' a group intermediate between the true bacteria and the true fungi.

(1) *Aerobic Actinomyces.* For description of the aerobic *Actinomyces*, see Jordan and Burrows, *Textbook of Bacteriology* (14th ed , 1945), pp. 599-620.

(2) *Anaerobic Actinomyces:* (a) *Actinomyces israeli.* If the typical morphology as described in Chapter IX has been seen in the direct examination of the material from the lesion, and in 0.2 per cent dextrose cooked heart medium or thioglycollate medium and in the surface growth on blood-agar plates, no further tests are necessary. In cultures heavily contaminated with additional micro-organisms, it is not always possible to isolate Actinomyces in pure culture. If the organisms are seen on direct smear and granules are present in the material, they should be washed in several changes of saline before being planted. Particular care should be exercised in the examination of the direct plates. Incubation in the anaerobic jar should be continued for 7 to 10 days. *Actinomyces israeli* may at times show feeble growth on the aerobic plate after several generations in laboratory media.

Animal pathogenicity tests are not indicated, as a negative result does not rule out the presence of Actinomyces in the suspected material.

Table V

Differential Characteristics of Cryptococcus Groups

Benham (*J Inf Dis.*)

Group	Colony	Description of Cells	Gas Formation in Sugars	Agglutination Serums			
				Group I	Group II	Group III	Group IV
1	Smooth or faintly convoluted, pasty white or cream	Round or oval, usually 1.5 to 4 5 by 2 5 to 4 5 μ. Buds single or in chains or groups. No capsules	+ or 0	++++	0	0	0
2	Convoluted, mucoid, cream to tan	Round or oval 3 to 6 5 by 4 5 to 6 5 μ Thick wall, faint capsule	0	0	++++	+	0
3	Smooth mucoid, cream to yellow or tan	Round 4 5 to 6 μ Thick wall, prominent capsule	0	0	+ or 0	++++	0
4	Smooth or convoluted, pink to red	Oval usually 2 5 to 4 by 3 to 5 μ. Thick wall, faint capsule	+ or 0	0	0	0	++++ or 0

For a discussion of these tests, see T. Rosebury, 'The Parasitic Actinomycetes and Other Filamentous Micro-organisms of the Mouth,' *Bact. Rev.* 8:189-223 (1944).

B. YEASTS

(1) *Cryptococcus neoformans* (*Cryptococcus hominis, Debaromyces neoformans*)· Cryptococci are differentiated from monilia by their failure to form mycelia. Cultures in corn-meal agar (Medium #33) should be made by cutting slits across a deep layer of the medium in a Petri dish with an infected needle. In 2 to 3 days, the hyphae of monilia grow out below the surface of the medium. The cryptococci will not form mycelia, regardless of the length of incubation.

Cryptococcus hominis belongs to Group 3. (See Table v.) In culture material, the gelatinous capsule may be demonstrated in dilute India ink films on glass slides. Strains from deep-seated lesions grow well at 37° C.; those from the skin grow poorly or not at all at incubator temperature. The pathogenic cryptococci will either produce lesions if injected intraperitoneally into rats or may be recovered from the peritoneal cavity 4 or 5 days after inoculation.

For detailed description of the cryptococci, see R. W. Benham, 'Cryptococci, Their Identification by Morphology and by Serology,' *J. Inf. Dis.* 57:255-74 (1935).

C. MOLDS AND MOLDLIKE FUNGI

See Jordan and Burrows, *Textbook of Bacteriology* (W. B. Saunders & Co, Philadelphia, 1945, 14th ed.), pages 620-50.

III. GUIDE TO MEDIA

All media referred to in the section on methods for bacteriologic examination of cultures from surgical infections are designated by number as well as by descriptive names:

PRELIMINARY IDENTIFICATION

Basic Media:

Beef infusion broth—Medium #2
Cooked heart medium—Medium #3
Brain medium—Medium #4
Thioglycollate medium—Medium #6
5 per cent blood beef infusion agar—Medium #15a

Selective Media:

Enteric bacilli.

Eosin-methylene blue agar—Medium #26
S-S agar—Medium #27

Tubercle bacilli:
- Petroff's medium–Medium #24
- Petragnani's medium–Medium #25

Diphtheria bacillus.
- Loeffler's serum medium–Medium #19
- Tellurite agar–Medium #22

Gonococci:
- Chocolate agar–Medium #20

Anaerobic nonsporulating bacilli:
- Enriched veal heart infusion broth–Medium #5
- Enriched veal heart infusion agar–Medium #16

Spirochetes:
- Hiss's serum water–Medium #11

Fungi:
- Maltose agar–Medium #32

IDENTIFICATION OF CULTURALLY PURE STRAINS

Acrobic Bacteria

Hemolytic streptococci:
- Dextrose beef infusion broth–Medium #2a

Staphylococci:
- Gelatin stabs–Medium #13
- Nitrate broth–Medium #9
- Bacto-phenol-red mannitol agar–Medium #17
- Loeffler's serum medium–Medium #19
- Milk agar–Medium #18

Neisseria·
- Carbohydrate ascitic fluid agar–Medium #23

Corynebacterium:
- Carbohydrate serum water–Medium #11a

Gram-negative nonsporulating bacilli:
- *Gelatin stabs–Medium #13*
- Bacto-peptone water–Medium #7
- Carbohydrate Bacto-peptone water–Medium #7a
- Clark-Lubs medium–Medium #8

Anaerobic Bacteria

Nonhemolytic streptococci:
- Iron milk–Medium #12
- Veillon's semisolid agar–Medium #31
- Maltose semisolid agar–*Medium #30a*

Staphylococci:
- Dextrose semisolid agar–Medium #30a

Veillonella:
- Dextrose semisolid agar–Medium #30a
- *Sugar-free semisolid agar (indol* test)–Medium #30
- Nitrate semisolid agar–Medium #28

Clostridia:
- 2 per cent glycerol broth—Medium #10
- Iron milk—Medium #12
- Iron gelatin—Medium #14
- Nitrate semisolid agar—Medium #28
- Sugar-free semisolid agar—Medium #30
- Carbohydrate semisolid agar—Medium #30a
- Lead acetate semisolid agar—Medium #29

Fungi:
- Corn meal agar—Medium #33
- Honey agar—Medium #34

Special Culture Media:
- For inhibition of swarming of Proteus: 6 per cent blood beef infusion agar—Medium #15c

IV. PREPARATION OF MEDIA

Medium #1—*Buffer Broth*

To 1 lb. of chopped beef add 1 liter of distilled water. Extract in icebox overnight. Boil 15 min Filter through filter paper.

Adjust pH to 6 Boil about 20 min. Add sufficient water to make volume 1 liter.

Add 10 gm. of neopeptone (Difco) and 4 gm. of dibasic sodium phosphate ($Na_2HPO_4 12H_2O$) Bring to a boil. Test pH and readjust to pH 8. (The final pH after sterilization should be 7.6-7.8) Filter through paper while hot. Dispense into sterile containers.

Sterilize in autoclave at 15 lb. pressure:
- Tubes and small flasks for 20 min.
- Flasks containing over 300 cc. for 30 min.

Medium #2—*Beef Infusion Broth*

Ingredients per liter of medium.

Chopped lean beef heart	1 lb.
Neopeptone (Difco)	10 gm.
Sodium chloride	5 gm.
Distilled water	1000 cc

Add chopped beef heart to distilled water. Extract in refrigerator overnight. Boil for 15 minutes, strain through cheesecloth. Add neopeptone and salt and stir until dissolved. Adjust pH to 7.8-8. Boil for 20 min., make up to original volume with distilled water, and filter through paper. Distribute in tubes or flasks as desired. Autoclave at 15 lb pressure: tubes and small flasks—20 min., large flasks (300 cc. and over)—30 min. Final pH should be 7.3.

Medium #2a—*Dextrose Infusion Broth*

Tube infusion broth (Medium #2) in 9-cc. amounts. Autoclave at 15 lb. pressure for 20 min. Add 1 cc. of a 10 per cent solution of dextrose in infusion

broth sterilized through a Berkefeldt or other bacterial filter. Incubate at 37° C. to check sterility.

Medium #3–*Cooked Heart Medium* (Holman's)
Ingredients per liter of medium:

Chopped beef heart	1 lb.
Neopeptone (Difco)	10 gm.
Sodium chloride	5 gm.
Distilled water	1000 cc.

Remove all fat, fascia, and blood vessels from fresh beef heart. Put through grinder. Stir chopped heart into distilled water and let stand in refrigerator overnight. Boil vigorously for 15 min. Strain through cheesecloth and restore to original volume with distilled water. Add neopeptone and salt and stir until dissolved.

Plain Meat Medium

Adjust pH to 8-8.2. Boil for 20 min., restore to original volume with distilled water, filter through paper until clear.

Dextrose Meat Medium

Adjust broth to pH 8.4. Boil for 20 min., restore to original volume with distilled water, filter through paper until clear. Add 0.2 per cent dextrose.

Wash the chopped cooked heart in a strainer under running water to remove fine particles, allow to drain, and squeeze out excess water. Distribute the cooked meat medium into 6 x ⅝-in. tubes. Fill the tubes ¼ full with heart particles. Add 6 cc. of broth. Then autoclave the tubes at 15-lb. pressure for 20 min. Final pH should be 7.4. If desired, add a piece of soft iron wire about 2 in long to the tubed medium before sterilization. The iron must not extend above the level of the medium.

Medium #4–*Brain Veal Heart Infusion Medium* (Robertson's)
Ingredients per liter of medium:

Chopped veal heart	1 lb.
Proteose peptone (Difco)	10 gm.
Sodium chloride	5 gm.
Distilled water	1000 cc.

Add chopped heart to water and allow mixture to stand in refrigerator overnight. Boil for 1 hr. Squeeze through cheesecloth. Add peptone and salt. Stir over flame until dissolved. Adjust pH to 7.6. Boil for 20 min., adjust to original volume with distilled water, and filter through paper. Check pH.

Fill 6 x ⅝-in. tubes ¼ full with chopped fresh calves' brain. Add 6 cc. of veal infusion broth. Autoclave at 15 lb. pressure for 20 min.

Medium #5–*Enriched Veal Heart Infusion Broth* (Anaerobic nonsporulating bacilli)

Extract 500 gm. of chopped veal heart in 1 liter of distilled water overnight in refrigerator. Add:

Neopeptone (Difco)	..	10 gm.
NaCl	..	5 gm.

Heat in double boiler at 70° C. until mixture turns dark brown. Add normal NaOH until slightly alkaline to litmus.

Steam in Arnold for 1 hr.

Strain through cheesecloth to remove meat, and filter broth through paper. Make up to 1 liter with distilled water, adjust pH to 7.6.

Boil for 15 min., make up to 1 liter, filter through paper, and distribute into flasks (100 cc. to 200 cc. each).

Sterilize in Arnold for 30 min. on 3 successive days.

When ready to use, add sterile solutions as follows:

Veal heart broth	100 cc.
{ Ascitic fluid	30 cc.
{ Blood	15 cc.
or	
Ascitic fluid	45 cc.
10 per cent cysteine hydrochloride	1.5 cc.
20 per cent dextrose solution	1.5 cc.
Gentian violet (1:1000 aqueous solution)	2.5 cc.

Adjust final pH to 7.5 and tube, using sterile precautions. If used for Gram-positive organisms, omit gentian violet.

Medium #6–*Thioglycollate Medium*

This medium may be purchased in a dried form requiring only the addition of water before tubing and sterilization.

The National Institute of Health [31] has circularized the following method of preparation:

l-cystine (Reagent)	0.75	gm.
Sodium chloride	2.5	gm.
Dextrose (Anhydrous)	5.0	gm.
Granular agar (Less than 15 per cent moisture by weight)	0.75	gm.
Water soluble extract of yeast	5.0	gm.
Pancreatic digest of casein	15.0	gm.
Distilled water	1000.0	ml.
Sodium thioglycollate or	0.5	gm.
Thioglycollic acid	0.3	ml.
0.10 per cent Solution of Resazurin (Freshly prepared)	1.0	ml.

Some difficulty may be experienced in getting the l-cystine into solution. (Hydrochloric acid may not be used as an aid in dissolving.) One method is to mix in a mortar all of the dry ingredients except the sodium thioglycollate (or the thioglycollic acid) in the order given in the table, thoroughly mixing each as it is added. Then stir in a portion of the water (previously heated), transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water or steam bath. Then add the sodium

thioglycollate or thioglycollic acid. Irrespective of the method used, preferable to add the sodium thioglycollate or thioglycollic acid after th *liminary heating. Adjust the reaction with sodium hydroxide to such a po* experience shows will result in a pH of 7.1 ± 0.1 in the *completed and* ized medium. Reheat, but do not boil, and filter (*only if needed for clarific* through a moistened paper filter, then add the resazurin solution. Distr into final containers and sterilize in the autoclave for 18-20 min. at 12 *to 123° C. (15 to 17 lbs. pressure).*

Storage of the Medium—After removal of the final container of medium the autoclave, cool promptly to 25° C. in order to set the agar. Store at 1 *to 30° C.* (preferably 20° C. to 30° C. as low temperature increases absor of oxygen from the atmosphere); avoid excessive light. If more than 30 pe of the uppermost portion of the medium has changed to a pinkish color, unsuitable for use. Under such circumstances one reheating in a boiling or steam bath is permissible in order to drive off the absorbed oxygen.

Medium #7—*Bacto-peptone Water*

To 1 liter of distilled water add 10 gm. of Bacto-peptone (Difco) and 5 of sodium chloride. Stir until dissolved. Adjust pH to 7.6 Boil for 15 Make up to original volume with distilled water. Filter through paper. and sterilize in the autoclave for 20 min. at 15 lb. pressure.

Medium #7a—*Carbohydrate Bacto-peptone Water*

Add 1 per cent Andrade's indicator to Bacto-peptone water. Tube in 4. amounts with inverted tubes (Dunham) for gas traps. Autoclave 20 mi 15 lb. pressure. Add 0.5 cc. of a 10 per cent solution of the desired c hydrate in Bacto-peptone water that has been sterilized by filtration thr a porcelain filter. Incubate at 37° C. overnight to check sterility.

Medium #8—*Clark-Lubs Medium* (Methyl-red and Voges-Proskauer tests)

To 1 liter of distilled water add

Peptone (Armour's)	5 gm.
Dipotassium phosphate	5 gm.
Dextrose	5 gm.

Dissolve, adjust pH to 7.6. Filter through paper, tube and autoclave 15 lb. pressure for 20 min.

Medium #9—*Nitrate Broth*

To 1 liter of water, add 10 gm. of neopeptone (Difco) and 1 gm. potassium nitrate—nitrite-free. Dissolve by heating. Filter through paper. T in 5 cc. amounts and sterilize by autoclaving at 15 lb pressure for 20 n

Medium #10—*Glycerol Broth* (Acrolein tests, *C. welchii*)

To 1 liter of beef infusion broth, add 20 cc. of glycerine. Tube 'deep' sterilize in Arnold for 30 min. on 3 successive days.

Small quantities may be prepared by adding 2 per cent glycerine to 100

of broth and filtering through Chamberland filter. Distribute into sterile tubes, taking care not to contaminate material.

Medium #11—*Hiss's Serum Water*

To 1 part clear sterile serum, add 3 parts of sterile distilled water. Distribute with sterile precautions into sterile tubes. Place in boiling water for 15 min. to destroy diastatic ferments. This method is preferable when serum water is only occasionally used. For a stock supply, add 3 parts of distilled water to 1 part of serum, tube deep, and sterilize in Arnold for 20 min. on 3 successive days.

For cultivation of spirochetes, a piece of sterile fresh rabbit kidney is added to the tubed serum water after sterilization.

Medium #11a—*Carbohydrate Serum Water* (for *C. diphtheriae*)[32]

Add 1 cc. of stock 1.6 per cent solution (alcoholic) of Bromcresol purple to 200 cc. of serum water (Medium #11). Add 1 per cent of desired sugars. Tube in 3-cc. amounts in serological tubes. Sterilize in Arnold for 30 min. on 3 successive days.

Medium #12—*Iron Milk* (Anaerobic organisms)[22]

Use fresh whole milk, mix cream by shaking. Tube in 10-cc. amounts. Add a piece of soft iron wire. Milk should completely cover wire. Autoclave at 115° C. for 20 min.

Medium #13—*Gelatin Stabs* (for staphylococci, aerobic Gram-negative bacilli)

To 1 liter of distilled water add 125 gm. of nutrient gelatin (Difco). Dissolve in double boiler to avoid scorching. Adjust pH to 7.3 and restore to original volume with distilled water Tube in 4-cc. amounts in serological tubes. Autoclave at 115° C. for 20 min.

Medium #14—*Iron Gelatin* (for *Clostridia*)[22]

To 1 liter of distilled water add 125 gm. of nutrient gelatin (Difco) and 1 gm. of dextrose. Dissolve in double boiler to avoid scorching. Adjust pH to 7.3. Tube in 10-cc. amounts in 6 x ⅝-in tubes. Add to each tube a strip of soft iron wire. Iron wire should be completely covered by gelatin. Autoclave at 115° C. for 20 min.

Medium #15—*Meat Infusion Agar* (Base for blood plates, slants, etc.)

To liter of beef heart infusion broth (Medium #2) add 20 gm. of granular agar (best grade) Dissolve. If necessary, readjust pH to 7.4. Autoclave at 120° C. for 20 min.

Medium #15a—*Blood-Agar Plates*

Melt agar, cool to 45-50° C Add 5 per cent sterile defibrinated or citrated blood, which has been warmed to 37° C. in water bath. Mix well before pouring plates. We prefer to use human blood. Plates should be incubated overnight at 37° C. to check sterility.

Medium #15b—*Blood-Agar Slants*

Pour blood agar (a) into tubes, slant, allow to harden, and incubate overnight to check sterility.

Medium #15c–*Six Per Cent Blood-Agar Plates* (for cultures containing Proteus group)

To 1 liter of beef heart infusion broth add 60 gm. of granular agar. Dissolve. If necessary readjust pH to 7.4. Autoclave at 120° C. for 20 minutes. Cool to 45-50° C. Add 5 per cent defibrinated or citrated blood, which has been warmed to 37° C. in the water bath. Pour plates. Incubate overnight to check sterility.

Medium #16–*Enriched Veal Heart Infusion Agar* (for anaerobic nonsporulating bacilli)

To 1 liter of veal heart infusion broth (Medium #5), add 30 gm. of granular or flake agar. Heat until agar is dissolved (Arnold steamer, 1 hr.). Make up to original volume with distilled water and filter through cotton if a clear agar is desired. Adjust pH to 7.8 if necessary. Distribute in flasks, 100 cc. or 200 cc. to a flask, and sterilize in Arnold steamer for 30 min on 3 successive days.

Before pouring plates, add the following sterile solutions to the melted agar, cooled to 45-50° C.:

Veal heart agar	100 cc.
{ Ascitic fluid	30 cc.
{ Blood	15 cc.
or	
Ascitic fluid	45 cc.
10 per cent cysteine HCl	1.5 cc.
20 per cent dextrose solution	1.5 cc.

Adjust final pH to 7.4. Solutions added to veal heart agar should be warmed to 37° C.

This enriched veal heart infusion agar has been found to favor the growth of the certain nonsporulating anaerobic organisms, especially the Gram-negative bacilli and actinomyces that grow poorly or not at all on the ordinary blood-agar plates.

Medium #17–*Bactophenol Red Mannitol Agar* (Staphylococci)

The dehydrated base requiring only the addition of water is satisfactory.

Medium #18–*Milk Agar* (for pigment production by Staphylococci)

Add 30 cc. of skim milk to 20 cc. of distilled water containing 1.5 gm. of agar. Autoclave at 115° C. for 20 min. Pour plates Incubate overnight to check sterility.

Medium #19–*Loeffler's Serum Medium* (for *C. diphtheriae*)[32]

To 3 parts of sterile beef or horse serum, add 1 part of meat infusion broth pH 6.8 to 7, containing 1 per cent dextrose. Mix by stirring, tube and inspissate on a slant, gently raising temperature until the serum is firmly coagulated. Sterilize for 20 min. in the Arnold on 3 successive days. After sterilization, seal tubes by dipping cotton plugs into melted paraffin and test for sterility by incubating for 24 hr. at 37° C. and 48 hr. at room temperature. Store at room temperature.

Medium #20–*Chocolate Agar* (for *Neisseria*)[32]

Remove all fat, fascia, and blood vessels from fresh beef hearts and put through grinder. For each 500 gm. of heart add 1 liter of distilled water. Allow to stand in refrigerator overnight. Strain and squeeze through coarse gauze. Add 10 gm. of proteose peptone #3 (Difco) per liter of infusion. Heat at 50° C. for 1 hr., then boil for 10 min. Strain through gauze and add 5 gm. of sodium chloride per liter. Adjust the pH to 7.6. Boil gently for 10 min. Dispense measured amounts into flasks and autoclave at 15 lb pressure for 15 min. Cool to 60° C. and add 5 per cent human or horse blood, place in water bath, and bring temperature up slowly to 80-85° C., rotating flask to produce even mixture. Cool to 50° C. and pour plates.

Medium #21–*Proteose 3 Hemoglobin Agar* (for *Neisseria*)

Media made from Bacto-proteose #3 Agar (Difco) and Bacto-hemoglobin (Difco) are satisfactory.

Medium #22–*Tellurite Agar* (for *C. diphtheriae*)[32]

It is not necessary to have a stock supply of this medium. When required, it may be made by melting 100 cc. of infusion agar (Medium #15) and cooling it to 50° C Add 10 cc. of citrated or defibrinated blood, and 10 cc. of a sterile 2 per cent potassium tellurite solution. Mix and pour into Petri dishes.

Medium #23–*Carbohydrate Ascitic Fluid Agar* (for *Neisseria*)[33]

Melt 100 cc. of sterile Douglas Agar, pH 7.4 to 7.8 in a flask. Cool to 48-50° C. With sterile pipettes add 20 cc. of sterile, bile-free ascitic fluid, 5 cc. of a sterile 20 per cent aqueous solution of the desired carbohydrate, and 1 cc. of sterile solution of Andrade's indicator. Pour into serological tubes, slant, and allow to harden. Incubate for 24 hr. at 37° C. to check sterility.

Douglas Agar·

Add 150 gm of minced lean beef muscle to 250 cc. of distilled water and heat to 80° C. Then add 250 cc of 0.8 per cent solution of anhydrous sodium carbonate. Cool to 45° C. Add 5 cc. Cole and Onslow's pancreatic extract and 5 cc. chloroform Incubate mixture at 37 5° C. for 6 hr. with frequent stirrings. Add 40 cc. N/1 hydrochloric acid Boil the mixture for 1 hr. Cool and filter through paper Add 10 gm. of granular agar for each 500 cc. of broth. Adjust pH to 7 6-7 8. Boil for 1 hr., filter through cotton. Dispense into flasks and autoclave at 15 lb pressure for 15 min.

Medium #24–*Petroff's Medium* (for *M. tuberculosis*)[32]

Infuse overnight in refrigerator 500 gm. of chopped beef or veal in 500 cc of a 15 per cent solution of glycerol in water. Place in a sterile press and collect the extract in a sterile container. Wash 28 eggs and immerse them in 70 per cent alcohol for 10 min Remove with sterile forceps, flame and break into a sterile beaker. Mix well by stirring and strain through sterile gauze into a sterile container. Add 1 part of meat juice to 2 parts of egg by volume. Add 1 per cent alcoholic solution of gentian violet so that the final concentration is 1:10,000. Mix well and tube Slant the tubes and inspissate, gently raising

temperature to about 85° C. Keep at this temperature until media is firmly coagulated. Sterilize for 20 min. on 3 consecutive days in Arnold. Test for sterility by 24 hr. incubation at 37° C., and an additional 48 hr. at room temperature.

Medium #25—*Petragnani's Medium* (for *M. tuberculosis*)[34]

Materials: 900 cc. skimmed milk; 36 gm. potato flour; 5 gm. peptone, 450 gm. sliced raw peeled potato; 24 hens' eggs; 70 cc. glycerol; 75 cc. 2 per cent aqueous solution malachite green; 2-qt. double boiler; sterile wire beaker with beater; sterile gauze, sterile glass funnel.

Procedure: Place in a double boiler 900 cc. of skimmed milk, 36 gm. of potato flour, and 5 gm. of peptone. Add 450 gm. of carefully selected sliced raw peeled potato. (Soak potatoes in 1:1000 $HgCl_2$ for ½ hour; wash in running water, peel, place shoe-string slices in distilled water until ready for weighing.) Weigh inner compartment of boiler. Heat the mixture, with constant stirring, for about 2 hr. Add sterile distilled water to make original weight. In the meantime place 24 washed eggs in 70 per cent alcohol for at least ½ hr. and then flame. Break the shells and transfer the contents into a sterile wire beaker fitted with a beater. After thorough agitation, add 70 cc. of glycerol and 75 cc. of 2 per cent malachite green (aqueous solution). Cool the milk-potato mixture to about 45° C., add to the glycerol-egg mixture, and thoroughly stir and mix. Then pass the stringy material through sterile gauze into a sterile dispensing funnel and distribute it into the sterile culture tubes, avoiding the formation of bubbles. Then heat this material in the inspissator at 80° C. for 1 hr., cool, and incubate for sterility. This medium can be sterilized in the autoclave at 5 lb. pressure for 1 hr. as follows.

Close all outlet valves. Heat very slowly the air trapped in the chamber Bring the jacket up to 5 lb. pressure very slowly (½ hour), then bring the chamber up to 5 lb. pressure and hold for 1 hr. Allow the autoclave to come down without opening the exhaust valves.

Medium #26—*Eosin-Methylene Blue Agar* (for coliform bacilli)[32]

Dehydrated Bacto-eosin-methylene blue agar (Difco) is satisfactory and requires only the addition of water before sterilization However, some laboratories prefer to make this medium.

To 1 liter of distilled water add 10 gm. of peptone, 2 gm. of dipotassium phosphate, and 15 gm. of granular agar. Boil until dissolved. Make up to original volume with distilled water. Dispense measured amounts into flasks and sterilize in the autoclave for 15 min. at 15 lb. pressure.

Just before using, melt the stock agar and add to each 100 cc. of the melted agar 5 cc. of 20 per cent sterile lactose solution, 2 cc. of 1 per cent sterile aqueous eosin (yellowish) solution and 2 cc. of 0.25 per cent sterile aqueous methylene blue. Mix well and pour into Petri dishes. Allow to harden and incubate overnight to test sterility. It is permissible to add all the ingredients to the stock agar at the time of preparation, place in flasks and sterilize. Decolorization of the medium occurs during sterilization but the color returns after it has cooled.

Medium #27—S-S *Agar* (for enteric pathogens)
The dehydrated medium (Difco) gives satisfactory results.

Medium #28—*Nitrate Semisolid Agar* (for *Clostridia* and *Veillonella*)[22]

Difco Tryptone .	5 gm.
Difco Neopeptone	5 gm.
Agar flakes	2 5 gm.
Distilled water	1 liter

Boil to dissolve and add water to make 1 liter. Adjust the pH to 7.3-7.4 and then add 1 gm. potassium nitrate, and 0 5 gm. of glucose. Dissolve and mix well. Tube 'deep.' Sterilize in autoclave at 120° C. for 20 min.

Medium #29—*Lead-Acetate Semisolid Agar* (for *Clostridia*)[23]

Proteose peptone	20 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Dextrose	1 gm.
Agar	2 gm.
Distilled water	1000 cc.

Dissolve, adjust to pH 7.6 and add 10 cc. of 2 per cent lead acetate. Tube 'deep.' Autoclave at 15 lb. pressure for 20 min.

Medium #30—*Sugar-Free Semisolid Agar* (base for fermentation media for anaerobic organisms, indol tests)[22]
To 1 liter of distilled water, add:

Neopeptone (Difco)	10 gm.
Tryptone (Difco)	10 gm.
Granular agar (best quality)	2.5 gm.

Dissolve by boiling and make up to original volume with distilled water. Adjust the pH to 7 3-7.4. Tube 'deep' Autoclave at 112° C. for 20 min.

Medium #30a—*Carbohydrate Semisolid Agar*
Divide above medium into lots and add 1 per cent of the desired sugars (saccharose, dextrose, lactose and salicin) Reserve one lot without sugar as a control Andrade's indicator may be added. However, this indicator is usually broken down by the metabolic products of the clostridia, and more reliable results are obtained if the pH of the medium is determined after the organisms have grown.

Medium #31—*Veillon's Semisolid Agar* (for anaerobic streptococci)[19]
To 40 cc of melted 2 per cent dextrose meat infusion agar, add 0 4 gm potassium nitrate and 30 cc of 0 5 per cent dextrose infusion broth. Tube in deep columns. Use sterile solutions and sterile tubes. Before using, place in boiling water for 20 to 30 min. to expel air, and cool rapidly to room temperature. Inoculate immediately. This is a soft rather than semisolid agar.

Medium #32—*Maltose Agar (Sabouraud's Medium)* (for fungi)

Maltose	40 gm.
Peptone	10 gm.
Agar	20 gm.
Distilled water	1000 cc.

Dissolve the agar in the water by boiling. Add the peptone and stir until dissolved Add the maltose and stir until dissolved Dispense into containers (200 cc. Erlenmeyer flasks or test tubes as desired) and autoclave at 15 lb. pressure for 30 min. The final reaction should be pH 5.2-5.4.

Medium #33—*Honey Agar* (for fungi)

Honey	60 gm
Peptone (Fairchild's)	10 gm.
Granular agar (Difco)	20 gm.
Distilled water	1000 cc.

Add honey and peptone to 55 cc. of the distilled water. Arnold for 1 hr. Dissolve agar in remaining 500 cc. of distilled water by heating in double boiler. Mix two preparations, adjust pH to 5.5 Dispense into flasks or tubes as desired. Autoclave at 15 lb. pressure for 30 min.

Medium #34—*Corn Meal Agar* (for fungi)

Corn meal	62.5 gm.
Granular agar (Difco)	19.0 gm.
Distilled water	1500 0 cc.

Add corn meal to 1000 cc. of the distilled water and boil for one hour. Filter through paper. Add agar to remaining 500 cc of water, heat in double boiler until dissolved. Mix two preparations, and measure. Make up to original volume with distilled water. Dispense into flasks or tubes as desired. Autoclave at 15 lb. pressure for 30 min.

V. STAINS AND INDICATORS

A. STAINS

1. *Gram Stain.* General Differentiation of Bacteria.
   (a) *Gram stain both for smears and films on glass and for tissue.*
   Hucker's modification [32] for smears or films on glass.
   Solution 1. Crystal violet: saturated alcoholic solution, 1 part, ammonium oxalate, 1 per cent aqueous, 4 parts.
   Solution 2. Gram's Iodine: iodine, 1 gm, potassium iodide 2 gm, distilled water 300 cc.
   Solution 3. Decolorizer. 95 per cent alcohol.
   Solution 4. Counterstain· saturated alcoholic solution of safranin 1 part, distilled water, 10 parts.

STAINING PROCEDURE. Stain with the crystal violet solution for 1 min. Wash in water. Apply iodine solution for 1 min. Wash in water. Decolorize in 95 per cent alcohol for 30 sec. or until violet dye fails to appear in the alcohol. Apply counterstain for 1 min. Wash in water and allow to dry without blotting.

Gram-positive organisms retain the crystal violet and stain dark blue; Gram-negative organisms stain pink.

(b) *Gram-Weigert Stain for Bacteria in Tissue Sections* (technic used in the Pathological Laboratory, Presbyterian Hospital, New York City).

Block tissue in paraffin in the usual way. Paraffin sections mounted on slides.

A. For Zenker-fixed tissue

1. Xylol, 5 min.
2. Xylol, 5 min.
3. Absolute alcohol, 5 min.
4. 95 per cent alcohol, 5 min.
5. 3-4 per cent iodine, 5 to 10 min.
6. 80 per cent alcohol, 5 min.
7. Wash in water.
8. 1 per cent sodium hyposulfate, 2 min.
9. Wash in water.

B. For formalin-fixed tissue

Omit 5, 8, and 9.

C. From this point with either A or B.

10. Sterling's crystal violet (filtered), 5 to 10 min (stain on slide)
11. Pour off and wash off with Gram's iodine.
12. Gram's iodine, 2 min.
13. Pour off and blot without washing.
14. Decolorize with aniline, 1 part, xylol 2 parts, on slide with drop bottle until section is pale violet.
15. Absolute alcohol, 1 second dip.
16. Wash in distilled water.
17. Blot.
18. Safranine 0.5 per cent aqueous solution, ½ min.
19. Wash in distilled water.
20. Blot.
21. Absolute alcohol direct, a few seconds.
22. Xylol, 5 min.
23. Balsam.

(NOTE Do not let slide dry after blotting.)

2. *Ziehl-Nielson Stain* (for Acid-fast Bacteria) [32]

Primary Stain:

1. Mix 10 cc of a saturated alcoholic solution of basic fuchsin with 90 cc. of a 5 per cent solution of phenol in distilled water.

2. Decolorizer: Three per cent hydrochloric acid in 95 per cent ethyl alcohol is satisfactory.
3. *Counterstain: Loeffler's alkaline methylene blue.*

STAINING PROCEDURE. Cover the smear or film with the primary carbolfuchsin *stain and heat gently until* steam appears on the surface. Allow to steam for 5 min., renew the stain repeatedly to prevent drying on the slide. If the slide is placed on a beaker of boiling water there will be no danger of drying. Wash with water. Decolorize the *acid alcohol until thin areas are colorless.* Wash with water, counterstain with Loeffler's alkaline methylene blue for one minute. *Wash with water and dry in air.* Acid-fast organisms appear red against a blue background. Nonacid-fast organisms are stained blue.

3. *Loeffler's Alkaline Methylene Blue* [32] (for C. diphtheriae)

Reagent:

Methylene blue (90 per cent dye content, certified) .	0.3 gm.
Ethyl alcohol (95 per cent) . . .	30.0 cc.
Potassium hydroxide (0.01 per cent aqueous solution) . .	100 0 cc.

Dissolve the methylene blue in the alcohol. Mix the methylene blue solution with the potassium hydroxide and filter.

STAINING PROCEDURE Allow the stain to act on the fixed film for at least 1 min. Wash in running tap water. Drain and dry without blotting.

4. *Hiss Method for Capsules.* [32]

Reagents:

1. Mix 10 cc. of saturated alcoholic solution of crystal violet or fuchsin with 90 cc. of distilled water. Undiluted Gram's crystal violet or *carbolfuchsin solutions may also be used.*
2. Copper sulfate, 20 per cent aqueous solution.

STAINING PROCEDURE. Prepare films from fresh material or by mixing cultures of organisms with animal serum, preferably beef serum. Dry in air but do not heat. Apply staining solution, heat until preparation begins to steam and allow to stain for 1 to 2 min. Wash off the stain with the copper sulfate solution Blot dry (do not wash) or examine under a wet cover slip. The capsule shows a lighter color than the body of the bacteria.

B. INDICATORS

1. *For Acid Production in Culture Media by Bacteria*

a. *Andrade's Indicator* [33]

Acid fuchsin	0.5 gm.
N/1 Sodium hydroxide	16 0 cc.
Distilled water	100.0 cc.

Dissolve the acid fuchsin in water and add the sodium hydroxide solution Sterilize in autoclave at 15 lb. pressure for 15 min This indicator is added to

give a 1 per cent concentration in the culture medium. It is colorless when alkaline, and pink when acid.

2. *For Anaerobiosis*

a. Equal quantities of the following solutions are combined just before using: 0.006 N. sodium hydroxide, 0.015 per cent aqueous methylene blue, sterile 6 per cent aqueous solution of dextrose. It is advisable to boil the tube with the indicator for 2 min. before placing it in the anaerobic jar to test for reduction of methylene blue when oxygen is expelled

b. Tube beef infusion broth pH 8 in 4 5-cc. amounts. Sterilize in autoclave at 15 lb. pressure for 20 min. Add 0.5 cc. of a 20 per cent dextrose solution sterilized by filtration through a Berkefeldt filter and 1 drop of sterile 0.5 per cent aqueous solution of methylene blue.

VI. SPECIAL TECHNICS

A. TECHNICS FOR PRODUCING AN ANAEROBIC ENVIRONMENT

The growth of anaerobic organisms is dependent on the removal of free oxygen from the bacterial environment and the production of the proper oxidation-reduction potential in the medium.

1. *Liquid and Semisolid Media*

These media should be tubed 'deep.' Free oxygen may be driven off from liquid or semisolid media by mechanical expulsion, that is, boiling. If these media are cooled rapidly before there has been time for re-absorption of air, multiplication of the anaerobic bacteria will occur in the lower levels of the medium. The addition of 0.1 per cent agar to a broth base minimizes convection currents, and these semisolid media are widely used. The addition of fresh or cooked tissue to a broth (that is, cooked heart medium) is of great value, since both the unsaturated fatty acids of the lipins and the glutathione content of the tissues co-operate in producing the necessary bacterial environment, the one through the removal of free oxygen, the other through the production of the proper oxidation-reduction potential Reducing substances such as sodium thioglycollate or thioglycollic acid may be added to the medium.

2. *Solid Media*

A. ABSORPTION OF OXYGEN

The rapid absorption of oxygen by pyrogallic acid is used as a means of ridding the atmosphere of oxygen in tubes containing agar slants, in single Petri dishes, and in large jars or desiccators.

(1) *Agar Slants (Wright's Method)*· The cotton stopper of the tube is pushed down to within one inch of the top of the test tube. Pyrogallic

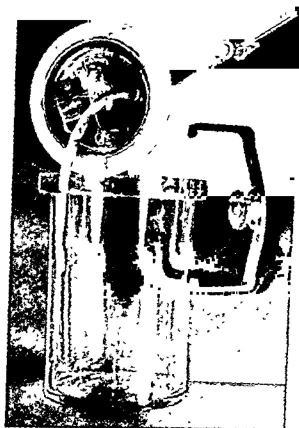


FIG. 5. Our modification of the MacIntosh-Fildes jar. This jar has given satisfactory service in our laboratory for over twenty years. With it anaerobic cultivation is obtained almost as easily as aerobic cultivation.

The inoculated tubes and plates are placed in the jar together with an indicator (see under special indicators). The copper basket is heated in a gas flame and hung on the hook on the underside of the lid. This basket should not come in contact with the cover of a Petri dish as the heat generated is sufficient to crack the dish. The lid is now firmly pressed into place. The hydrogen is turned on and allowed to flow slowly into the jar. Combustion starts immediately and is evidenced by moisture forming on the side of the jar. The lid gradually becomes warm. After combustion has become well-established, the clamp should be placed on the jar and tightened. Hydrogen should be allowed to flow in until the valve on the tank registers 2½ lb. pressure within the closed system. If the hydrogen has been allowed to run in slowly, this will take from 20 to 30 minutes and the oxygen will be completely used up as evidenced by the cooling of the lid of the jar. The jar is then disconnected and placed in the incubator. The complete decolorization of the indicator, after 10 to 12 hours, shows complete or satisfactory anaerobiosis. The red blood cells in the blood-agar plates become a very dark red, showing that oxygen has been removed from the agar medium. These plates return to their original color when removed from the jar.

B. TESTS FOR SENSITIVITY TO ANTIBIOTICS

1. *Methods for Penicillin*

A. FILTER-PAPER DISCS

This method has the advantage that a test for sensitivity to penicillin may be included as a routine procedure on the direct platings of culture material.

The blood-agar plates are streaked with the swab or other culture material. Filter-paper discs ½ in. in diameter may be cut out of Whatman's filter paper #1 and sterilized by dry heat. The disc is moistened in a tube containing a standard penicillin solution (2 units per cc.) in buffer solution at pH 7 and the excess drained off against the side of the tube. The disc is then placed on a part of the plates that has received a heavy inoculum of the culture material. If heavier discs are used, such as Schleicher and Schuell analytical filter-paper discs #740-E, the disc is placed on the plate and 0.1 cc. of the standard 2 units per cc. penicillin solution is pipetted immediately onto the disc. Care must be taken that the disc does not absorb moisture from the plate before the penicillin is pipetted onto the disc. Metal or glass cylinders may be used instead of discs (Fig. 6).

When the plates are examined after 18 to 24 hours' incubation at 37° C., there will be a zone of inhibition of growth of organisms sensitive to penicillin around the filter-paper disc. Resistant organisms will grow up to and even under the filter-paper disc (Fig. 7). Too great reliance should not be placed on this test if the growth of the seemingly sensitive organisms on the remainder of the plate is scant with widely separated colonies. Such organisms should be retested after isolation in pure culture by streaking a large loopful of an overnight broth culture of the strain across a blood-agar plate and placing a filter-paper disc moistened in the standard 2 unit per cc. solution of penicillin on the central portion of the streak. If there is a zone of inhibition of growth adjacent to the filter paper, the organism may be considered penicillin sensitive. If there is no zone of inhibition, a more exact determination of the degree of penicillin sensitivity or resistance should be determined by the serial dilution in broth method. All penicillin-resistant staphylococci, micrococci, aerobic Gram-negative bacilli, and subtilis strains should be tested for penicillinase production.

B. DILUTION METHODS

(1) *Test of Sensitivity of Unknown Strain in Relation to That of Standard Organism, C203Mv, a Group A Hemolytic Streptococcus (as Used in the Penicillin Laboratory of the Presbyterian Hospital, New York City):* A stock solution of penicillin containing a known number of Oxford

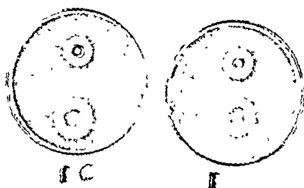


FIG. 6. Inhibition of growth of staphylococcus in agar pour plates around pennicylinders (upper) and filter paper discs (lower). Penicillin concentration: upper, 1 unit per cc, lower, 2 units per cc.

units per mg. is made up in 0.85 per cent saline, 1 mg./ml. Sufficient solution for 1 to 2 days may be kept in the refrigerator. If a large supply of stock solution is made up, it is distributed in small tubes and kept in dry ice; while frozen the penicillin remains stable. Immediately before the setting up of tests, a saline solution containing 100 units/ml. is made from the stock solution, e.g.

1 ml. stock solution containing 790 units/ml. + 6 9 ml.

saline = solution containing 100 units/ml.

From this solution are prepared two sets of serial dilutions in broth—10 units/ml. to 0.00156 u/ml.—as follows:

Tube	1-0 2 ml	100	u/ml. penicillin	+ 1.8 ml.	broth	contains	10	u/ml
"	2-0 2 ml.	10	u/ml	"	+ 1 8 ml	"	"	1	u/ml
"	3-0 4 ml	1	u/ml.	"	+ 3 6 ml.	"	"	0 1	u/ml
"	4-1 8 ml.	0.1	u/ml	"	+ 1.8 ml.	"	"	0 05	u/ml
"	5-1.8 ml.	0 05	u/ml.	"	+ 1 8 ml.	"	"	0.025	u/ml.
"	6-1 8 ml.	0 025	u/ml	"	+ 1 8 ml	"	"	0 0125	u/ml.
"	7-1 8 ml.	0 0125	u/ml	"	+ 1 8 ml	"	"	0.00625	u/ml
"	8-1.8 ml	0 00625	u/ml	"	+ 1.8 ml	"	"	0 003125	u/ml
"	9-1.8 ml	0 003125	u/ml.	"	+ 1.8 ml	"	"	0.0015625	u/ml

1 8 ml. of highest dilution is discarded



FIG. 7 Filter paper discs on two staphylococcus cultures, one sensitive and the other resistant to penicillin.

To each tube of one series is added 0.2 ml. of a 10^{-2} dilution of a 15-16 hour blood broth culture of C203Mv, making the final dilution of culture 10^{-3} and the total volume in each tube (except 2nd and 3rd) 2 ml. To each tube of the second series is added 0.2 ml. of the unknown culture so diluted as to be comparable in density with C203Mv. If an organism grows slowly or lightly, two seedings, 10^{-1} and 10^{-2}, are tested. If an organism requires blood for good growth, a drop of sterile rabbit's blood is added to each tube of the test. Tubes are shaken and incubated 18 to 24 hours. Pour plates of the two organisms diluted 10^{-7} and 10^{-8} are made for colony counts.

For convenience, complete absence of turbidity is interpreted as complete *inhibition of growth. The 'relative sensitivity' of a strain is* determined by comparing the highest dilution of penicillin inhibiting growth in the two titrations. Growth of the standard organism, C203Mv, in a dilution of 10^{-3}, which contains on an average 500,000 organisms/ml., is inhibited by 0.025 u/ml. If the end point of inhibition in the titration with the unknown strain occurs in the tube containing 0.1 u/ml., the penicillin sensitivity of the unknown strain is said to be ¼ that of the standard organism.

Because of the intervals between dilutions, this method indicates only roughly the amount of penicillin required for inhibiting growth of an organism. If a definite guide to dosage is desired, a more accurate determination of sensitivity may be made by the following method.

(2) *Test of Sensitivity of Strain in Terms of Units of Penicillin Required for Inhibition* The highest dilution of penicillin that inhibited growth of the unknown strain in the first test is titrated, in duplicate, in amounts decreasing by tenths, from 1 ml. to 0 1 ml., the volume is made up to 1 ml. with broth and 0.1 ml. of culture in a dilution comparable with 10^{-4} of C203Mv is added. If an organism grows slowly or delicately, tests of more than one seeding are set up.

The validity of the test is controlled by a titration, in duplicate, of a penicillin solution containing 0.025 u/ml. with C203Mv as the test organism. Six tubes are sufficient, 0.7-0 2 ml (see table). This titration is seeded with a 10^{-4} dilution of C203Mv, 0.1 ml. per tube—a seeding of approximately 5000 organisms.

The titrations are incubated 24 hours or longer if necessary for growth in the control tube containing 0 005 u. penicillin The end point of inhibition of C203Mv should be in the tube containing 0.01 u., but a variation of 0 0075 u. to 0.0125 u is within the margin of error of the test.

A strain inhibited by 0.1 u penicillin in the preliminary test may have an inhibition end point of 0 06 u in test (2), and in that case its

TABLE VI

PENICILLIN CONTENT OF EACH TUBE IN OXFORD UNITS/ML.

AMOUNT OF DIL. PENIC. PER TUBE IN ML.	PENICILLIN DILUTION TITRATED					
	10 Units	1 Unit	0.1 Unit	0 05 Unit	0 025 Unit	0 0125 Unit
1	10	1	0.1	0 05	0 025	0 0125
0.9	9	0 9	0 09	0 045	0 0225	0 01125
0 8	8	0 8	0 08	0 04	0 02	0 01
0.7	7	0.7	0 07	0 035	0 0175	0 00875
0 6	6	0 6	0 06	0 03	0.015	0 0075
0 5	5	0 5	0 05	0 025	0 0125	0 00625
0.4	4	0 4	0 04	0 02	0 01	0 005
0 3	3	0 3	0 03	0 015	0 0075	0 00375
0.2	2	0 2	0 02	0 01	0 005	0 0025
0 1	1	0 1	0 01	0 005	0 0025	0 00125

penicillin sensitivity is reported as follows: 0.06 unit penicillin is required for inhibition of growth.

C. TEST FOR PENICILLINASE PRODUCTION

Some bacteria produce an enzyme penicillinase, which destroys penicillin.[35] The penicillin-resistant staphylococci, micrococci, aerobic Gram-negative bacilli, and subtilis strains are the chief offenders.[36-7]

To 1 ml. of a 24- to 48-hour culture of the test strain in beef infusion broth is added 1 cc. of a standard penicillin solution containing 40 units of penicillin per ml. in buffer solution, pH 7. A control for the titer of the penicillin and its stability under the assay conditions is also set up, that is, 1 ml. of beef infusion broth plus 1 ml. of the standard penicillin solution. Both tubes are incubated at 37° C. in a water bath for 2 hr. The tube containing the test culture is then diluted to a calculated 10, 5, and 1 unit of penicillin per ml., the control tube to 1, 0.5 and 0.25 units per ml. These dilutions are assayed against the Oxford strain of *Staphylococcus aureus* by the penicylinder-agar plate method.[38, 9] By a comparison of the zones of inhibition from the test preparation and the control preparation it can be determined whether there has been complete, partial, or no destruction of the penicillin by the test culture.

2. *Methods for Streptomycin*

A. BROTH DILUTION

Various factors will influence the apparent sensitivity of organisms to streptomycin *in vitro*. Among these are age of culture, density of culture, species of micro-organisms, medium used, pH, buffer concentration, etc.

The method described below is used in the laboratories of the Presbyterian Hospital in New York City.

Beef infusion buffered broth (Medium #1) has been found satisfactory. Use 6-hour cultures prepared from overnight cultures by inoculating 8 cc. of sterile broth with 0.1-0.25 cc. of overnight culture. Incubate at 37° C. Turbidity at end of 6 hours should be such as to give 78 per cent light transmission. If too light, culture must be seeded more heavily. If too dense, culture must be diluted to 78 per cent transmission before use.

Prepare sterile solution of streptomycin containing 50 micrograms per cc. in sterile water. Into a series of 12 tubes, pipette the following amounts of streptomycin solution:

0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09,
0.1, 0.15, 0.20 cc

Bring to total volume of 0.5 cc. with sterile broth. Seed each tube with 0.5 cc. of a 1-1000 dilution of the unknown culture, prepared as described above. Mix well. Incubate at 37° C. for 72 hours.

The end point is the least amount of streptomycin that will cause complete inhibition of growth after 72 hours of incubation, as determined by the naked eye. Readings are made at 24 and 48 hours as well as 72 hours to determine the degree of bacteriostasis.

In spite of attempts to control age and density of culture, and composition of medium, apparent sensitivity of a given culture may vary slightly from day to day. For this reason, a standard strain such as the Waksman strain of *E. coli* is tested for sensitivity daily and the sensitivity of unknown organisms expressed in terms of a ratio,

$$\text{i.e. } \frac{\text{sensitivity of unknown}}{\text{sensitivity of } E.\ coli\ W \text{ on same day}} = \text{Resistance of unknown.}$$

The sensitivity of *E. coli* W in the above medium has been found to vary from 2 to 7.5 micrograms per cc. In a less rich medium as used by Waksman, sensitivity is greater and ranged from approximately 0.5 to 2.0 micrograms.

C. Determinations of Concentrations of Antibiotics in Body Fluids and Exudates

1. *Penicillin*[40] *and Bacitracin*[41]

A. MATERIAL

All samples of blood for assay should be collected in sterile tubes and allowed to clot. The serum is withdrawn and stored in the refrigerator until the test is run. If material such as urine, pleural exudates, joint fluids, and so forth is known to be or suspected to be contaminated, it should be sterilized by filtration through a Berkefeldt or Chamberland filter and then kept at icebox temperature until tested, since bacterial contaminants may destroy penicillin.

B. TEST

A twofold serial dilution of 0.2 cc. of the unknown is made in 0.2 cc. beef infusion broth through 10 to 12 dilutions. A similar series of dilutions is made using 0.2 cc. of a standard penicillin solution in physiological saline known to contain 20 units of penicillin per cc. To each tube in both series in added 0.5 cc of infusion broth containing 1 per cent washed red blood cells and 1000 to 10,000 Group A hemolytic streptococci. All tubes are incubated at 37° C. for 18 hours.

C. READING OF TEST

The cultures are examined for hemolysis as evidence of the multiplication of the streptococci. Cultures showing no hemolysis are usually sterile, but the tubes on either side of the apparent end point in each series should be streaked on blood-agar plates to check the presence or absence of viable bacteria. The concentration of penicillin in the 0.2 ml. of the unknown is determined by comparison of the end point of this series with the end point in the control series, where the amount penicillin in each tube is known.

2. *Streptomycin* (Methods used in the Penicillin Laboratory of the Presbyterian Hospital, New York City)

A. IN SERUM

Equipment: 5-cc., 1-cc., 0.2-cc. pipettes
Sterile normal serum (horse or human)
Patient's serum (3-4 cc. is adequate)

Using sterile 1-cc. pipettes, measure the following amounts of patient's serum into sterile tubes: 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.10 cc. If it is expected that level will be high you may wish to omit some

of the larger amounts and (with 0.2-cc. pipette) measure into sterile tubes 0 09, 0.08, 0.07, 0.06, 0 05, 0.04, 0.03, 0.02, 0.01, 0 0 cc.

If no estimate of the range is possible, 19 tubes will cover the entire range. Using 1-cc. and 0.2-cc. pipettes as indicated, bring volume in each tube to 0.5 cc. by adding sterile normal serum.

Seed each tube with 0 5 cc. of broth culture (see below).

Incubate 37° C.—30 to 72 hours or until end point does not change on further incubation. Read end point and calculate units per cc. from standard as in case of penicillin levels.

(1) *Standard:* Use streptomycin of known micrograms per milligram. Make up to 50 micrograms per cc. in normal sterile serum. Using 0.2-cc. pipettes, measure 0.20, 0.15, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05, 0 04, 0 03, 0 02, 0.01, 0.0 cc. to each of 13 tubes. Make up to 0 5 cc. by adding sterile normal serum. Seed each with 0.5 cc. of broth culture (see below).

Incubate 37° C.—30 to 72 hours or until end point does not change.

End point is least amount (mcgs./cc.) that causes complete and permanent inhibition of growth.

(2) *Preparation of Culture:* It is important to use an organism of maximal sensitivity to streptomycin, since the more sensitive the culture, the lower will be the blood levels obtained. *Escherichia coli* (Waksman) or the F.D.A strain (P.C.I. 602) of *Klebsiella pneumoniae* will give satisfactory results.

Use a 6-hour culture in buffered broth (Medium #1) prepared by seeding 8 cc. of the buffered broth with 0 1 to 0.4 cc. of an 18-hour buffered broth culture. The size of the inoculum will depend on the rate of growth of the organism used.

Dilute the 6-hour culture with buffered broth to yield a density equivalent to $BaSO_4$ #1 Standard or to 78 per cent transmission of light on Photovolt #400 Lumetron (this is equivalent to 300 million organisms per cc , if other turbidity standards are used).

Then dilute to 10^{-3} and use this 10^{-3} dilution to inoculate blood levels tubes.

Tubes should be inoculated immediately as soon as dilutions have been made.

Age of culture, density of culture, number of organisms per cc. (note 2 cultures of different ages may show same number of organisms but different densities), buffer content of medium, pH, etc., all influence sensitivity of organism to streptomycin and will interfere with blood level determinations if not properly controlled.

If a medium different from that described above is used, it may be necessary to adjust the above culture dilutions accordingly. *Note.* If end point for standard is low (i.e. 0.5-1.0 mcg./cc.) and serum

level is 50-100 mcgs. per cc., it may be necessary to repeat, using a slightly more dense culture. A 10^{-2} dilution in place of 10^{-3} will usually shift the end point enough to allow for an accurate determination of serum level.

B. IN SPINAL FLUID

Use same procedure as for serum, but bring to volume with sterile normal spinal fluid rather than serum, and dilute standard in normal spinal fluid rather than in serum.

C. IN URINE

Use essentially the same procedure as for serum, but bring to volume with sterile broth rather than with urine. Serum may be used in place of the broth and tends to slow up the growth of the organisms—making for greater sensitivity. Serum is not necessary, however. Urine enhances the growth of the Gram negatives to the extent that they appear almost insensitive if too high a concentration of urine is used.

As urine level will be high, urine should be diluted and set up as follows:

Heat urine 80° C.—30 minutes to sterilize. Then dilute portions of it 1-5, 1-20, and 1-40 in broth or normal serum.

Set up tubes containing the following amounts:

Undiluted: 0.1, 0 09, 0 08, 0 07, 0.06, 0.05, 0.04, 0 03, 0.02, 0.01 cc.
1-5: 0 05, 0.04, 0.03, 0.02, 0.01 cc.
1-20: 0.05, 0.04, 0.03, 0.02, 0.01 cc.
1-40: 0.05, 0.04, 0 03, 0.02, 0.01 cc.

Bring tubes to 0 5-cc. volume with broth or serum, depending on which was used to dilute urine

Inoculate with 0.5-cc. culture as for serum levels. (Note that amount of urine per tube is very low in most instances. Except in the case of pre-therapy or posttherapy urines, the undiluted series can be omitted.)

For urine levels, dilute standard in broth.

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CHAPTER XI

Bacterial Entrance into the Human Body

THE HUMAN integument is a fairly impermeable and impenetrable layer varying considerably in thickness in different parts of the body. Everywhere it is perforated by the ducts of sebaceous and sweat glands, as well as hair follicles, which are pathways by which certain bacteria are able to enter the interior of the body. At the orifices of the body, the skin meets mucous membrane that lines the so-called inner surfaces of the respiratory, alimentary, and genitourinary tracts, as well as the inner surfaces of the eyelids and outer surface of the eyeballs.

In somewhat different manner, glands of varying sizes project inward from the inner body surfaces and secrete their specific substances through their ducts upon the surface of the mucous membrane. This latter group includes the salivary glands, the mucous glands of the mouth and esophagus, the liver and pancreas, the kidneys, and certain minor glands of the stomach, intestine, and vagina. There are also certain so-called closed cavities of the body with serous linings, namely the joints, the bursae, the tendon sheaths, the pleurae, the cavity containing the brain and cord, and the peritoneum. The last has a communication with the external surfaces in the female through the Fallopian tubes.

Normally, the external surface of the body is in contact with countless organisms. This is true, also, of the gastrointestinal tract except for certain periods of digestion when the stomach and the upper part of the duodenum are free from viable organisms. It is true only of the upper part of the respiratory tract, for except under abnormal circumstances no considerable number of viable bacteria are found in the bronchi or alveoli of the lungs, although from time to time a few organisms doubtless penetrate these regions. Likewise, the urinary tract is normally free from organisms except near the orifices, and the genital tract in men is free to the same degree. In women, contamination does not usually extend beyond the cervix of the uterus. The closed cavities are normally free from bacteria.

The manner of entrance and spread of bacteria into and through the human body can be demonstrated only by the complete examination of autopsy material. Even then details often escape the most careful

observers. Some of these facts have been brought out by a review of the case records and a study of the sections from the cases presented in this chapter and in Chapter XIII. Death from infection has been materially reduced since the sulfonamides and penicillin have been introduced. We must go back a decade or more to get illustrative material. The author wishes to express here his very great appreciation to the clinicians who had the hospital care of these patients and to the pathologists who gave him such free access to the material.

I. THE SKIN

The glands on the surface of the body tend to discharge organisms deposited in their openings because of the more or less constant flow of their secretion. If for any reason the openings become plugged, organisms may be retained that, if able to grow and multiply, may so injure the lining epithelium of the glands as to break through the surface into the deeper tissues. This happens very frequently, and superficial infections, such as furuncles, are certainly produced in this way. These infections are so common that they hardly need illustration. They are nearly always staphylococcal, but lesions of anthrax or glanders and occasionally hemolytic streptococcal infections are produced without any known break in the skin, as is illustrated by the following two cases seen and treated several years before the advent of the sulfonamides and antibiotics:

Case I Name R.M.

HISTORY. A pathologist was performing an autopsy on a man who had died of primary hemolytic streptococcal peritonitis He wore long gauntlet gloves reaching halfway up the forearm, but while removing the abdominal contents, he was aware that his forearms above the gloves became smeared with the peritoneal exudate. Two days later, three or four furuncles developed in this area from which a pure culture of hemolytic streptococcus was obtained Subsequently, the infection traveled to the axillary lymph glands where, after several incisions, it could only be eradicated finally by complete block dissection of the gland mass.

Case II. Name K.T S.

HISTORY. A housewife developed redness, swelling, and tenderness on the inner side of the right arm without any known break in the skin. On the fifth day, a portion of this area became black, and two days later the upper margin separated and discharged seropurulent exudate. On admission to the hospital on the seventh day, she was prostrated with a temperature of 103 3° F. The arm showed a gangrenous area of skin on the inner side of the arm and earlier stages of gangrene lower down with edema and redness fading off into the surrounding skin. Cultures revealed only hemolytic streptococci. It was a typical case of hemolytic streptococcal gangrene. Several incisions were made

COURSE. At operation, a large carcinoma of the pylorus of the stomach was found, with extensive peritoneal metastases. A posterior gastroenterostomy was performed. The postoperative course was uneventful until the sixth day, when the temperature rose slightly, and for eleven days it ranged between 99.6° F. and 101.2° F. The wound showed very little tendency to heal, but did not reveal any evidence of inflammation. The stitches were finally removed on the twenty-second day, and the wound appeared to be healed. During the next week, her temperature remained normal, but she failed rapidly and died on the twenty-eighth day after operation.

AUTOPSY. The finding of particular interest in this case from the bacteriological point of view was as follows: On opening the recent operative wound in the upper portion of the rectus muscle beneath the sheath, a brownish red, semifluid mass was found. A few drops of greenish yellow pus were present around a piece of suture material. Cultures from this area yielded hemolytic streptococcus. Microscopically, the suture material was found to be surrounded by pus cells in which there were a few Gram-positive diplococci.

This case illustrates the entrance of bacteria at the time of operation, and their growth in the neighborhood of suture material and extravasated blood. In this case, they were relatively nonvirulent but persisted in the wound of the debilitated patient for about a month (Fig. 1).

Case IV: Name W.M. *History no.* 333204.

HISTORY. A doctor was performing an operation on a young girl with a hemolytic streptococcal empyema. During the course of the operation, he broke open a sterile ampule of novocain. The glass penetrated his glove and produced a small puncture hole in the anterior surface of the middle phalanx of the right index finger. On the fifth day, he noted a little pus exuding from the puncture wound. Six hours later, his finger became painful and woke him out of his sleep. A colleague made an incision through the puncture wound. No pus was found. This did not relieve the situation so the doctor entered the hospital. He then suffered a violent chill. Blood culture was negative. Skin flaps were made on the middle and proximal volar surfaces, but next day the distal half of the finger became gangrenous. Blood culture taken on the second day revealed a hemolytic streptococcus, but from the finger, both a hemolytic streptococcus and a *Staphylococcus aureus*. The finger was amputated, and the basilic and cephalic veins ligated. Staphylococcus bacteriophage was given both locally and systemically. The infection then came under control, although the proximal end of the tendon sheath developed a pocket of pus and a hemolytic streptococcal abscess developed in the deltoid region following a hypodermic injection at that site.

The upper layers of the skin epithelium are dead, dried, flattened cells that have lost their nuclei. They have been pushed up by the cells immediately beneath them and have been shut off from their supply of nourishment. The dryness and the keratinization that takes place in them, together produce a strong barrier against the passage of organisms. It is

unlikely that organisms ever pass through this hornified covering, but if this layer be removed from any cause, down to the level where the cells are alive, and where the body fluids are circulating, bacteria implanted at these points may enter the deeper tissues. If they are carried down through this hornified layer by means of the ducts of the sebaceous or sweat glands, they may reach a level of very much less resistance to their penetration.

Bacteria may enter, also, through pathological sinus openings. The author has seen a case of acute parotitis that seemed to have been caused by bacteria entering a chronic salivary fistula.

II. THE RESPIRATORY TRACT

In the upper respiratory tract, organisms are almost invariably present. These may be demonstrated on the surface of the mucous membrane of the nose. The degree to which they penetrate the mucosa varies considerably, according to the physical condition of the mucous membrane and the nature of the organisms. The frequency of inflammations of the nasal mucous membrane, as in the common cold, indicates that at times organisms penetrate at least far enough to call forth the exudation of fluid and cells. A nervous reflex to mechanical or thermal irritation of these surfaces also results in a flow of secretion, as well as congestion and edema of the mucous membrane. Organisms normally lying on the surface in a perfectly indifferent manner may then find the physical conditions so altered that they may penetrate into the deeper parts. They may also be carried into the deeper parts by phagocytic cells that come out to meet them. If atropin is administered in the early stages of exudation, the drying of the mucous membrane that results will frequently prevent an infection that would otherwise occur. Mechanical abnormalities, such as adenoid tissue, polypoid growths, or the contact of turbinates with the septum, may alter the condition of the mucous membrane so as to favor the entrance of organisms into the deeper tissues. When air is inhaled through the nose, bacteria are carried in through the nostrils, but the current of air is immediately subjected to variations in direction, which tend to cause any particles within it to catch upon the moist surfaces rather than to pass down through the larynx, trachea, and bronchi. With mouth breathing, there is of course more chance for direct inhalation In either case, inspired air may occasionally carry organisms down into the lower part of the lungs. When the secretions are excessive, droplets may be inspired, particularly with labored breathing or when the laryngeal or tracheal reflexes are lost, as, for example, under anesthesia. When there is infection of the naso-

pharyngeal mucous membrane, organisms may spread downward either directly or through the lymphatics.[1] Cases v and vi in this chapter and also Case vii in Chapter xiii illustrate these types of infection.

Under surgical anesthesia, the cough reflex is lost so that there is very little protection against the passage of mucus and its contained bacterial flora down into the trachea and bronchi. Certain anesthetics, such as ether, cause an increased secretion of mucus from the glands lining the tract and, in the initial stages at least, increase the amplitude of respiration. The position of the patient's head with relation to the lungs brings gravity into play. Many nose and throat operations are done with the head down to prevent the gravitation of blood and mucus into the lungs Sometimes it is necessary for the anesthetist to insert a laryngoscope into the trachea and then a tube, which is frequently left in place all during anesthesia. This carries mouth organisms in a certain distance, and they may go even farther under their own power. When coming out of an anesthesia, vomiting is not uncommon. Fortunately, the cough reflex returns at about the same time, but occasionally vomitus is aspirated with fatal consequences. Mucus may act as a plug in the bronchi and result in atelectasis. If such a plug is not quickly expelled, the contained bacteria may invade the neighboring lungs and cause a bronchial or even a lobar pneumonia.[2, 3]

Some years ago, the author made some observations on the spread of bacteria into the lungs when they had been introduced into the nasopharynx of rabbits under anesthesia. Paradoxically enough, in that animal the organisms were more often found at the base of the lungs when the body was held at a 45° angle with head down rather than with head up. This condition seemed to be accounted for by the fact that in the head-down position, the abdominal contents crowded the diaphragm up toward the head, and the greater inspiratory effort necessary carried the bacteria quickly to the base through the foreshortened bronchial tree. This would not apply to human anatomy where gravity would probably be a much more important factor.[4]

Case V: Name T.R. Age 25. History no. 62626. Autopsy no. 9630.

HISTORY. The patient had a 'head cold' for one week prior to his entrance On the day before admission, he suddenly developed chills and fever, with pain in the right chest and cough with sputum. Malaise was profound, and he vomited repeatedly.

PHYSICAL EXAMINATION. The man was well developed and well nourished. His face was flushed, his lips dry, and his respirations rapid and shallow. The lungs revealed dullness at the right base, with fine crepitant râles, high-pitched bronchial breathing, and absent fremitus. His temperature was 102° F., pulse rate 128, and respirations 25. The heart action was regular, but the sounds



FIG 2 CASE V· Suppurative pneumococcus pleuritis. Thick pleural exudate over consolidated lung, showing the long fibrin strands and the dense cellular infiltration, × 50. *Insert* Pneumococci in the exudate, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

were of poor quality. The blood pressure was 75/50 The white blood count was 5900, with polymorphonuclears 75 per cent. Blood culture yielded pneumococcus Type I

COURSE. As soon as the organism was known, the patient was given 100 mils of Type I antipneumococcus serum intravenously—sulfonamide and penicillin were unknown at that time. On that day, his blood count was 4800, with polymorphonuclears 85 per cent Later in the day, 50 mils more of antipneumococcus serum were administered. On the third day, the white blood count fell to 2900, with polymorphonuclears 72 per cent. Consolidation spread to the left lower lobe. For the first three days, he was treated intensively with antipneumococcus serum, receiving 550 mils in all. On the fifth day, his white blood count rose to 21,500, with polymorphonuclears 83 per cent, and a blood culture was sterile. Nevertheless, his heart failed and he died on the seventh day after admission.

AUTOPSY. The thoracic cavity on the right side contained 800 mils of purulent exudate with a thick, fibrinous layer over the lung and between the lobes. On the left side were 200 mils of slightly blood-tinged, clear fluid. The right lung showed a consolidation of the right middle and right lower lobes The left lower lobe was similarly involved, but in an earlier stage The pericardium contained a small amount of clear fluid. The mitral valve showed

delicate vegetations. The rest of the autopsy was negative. The pus from the pleural cavity cultured pneumococcus Type I. The microscopic examination showed the lung alveoli to be filled with an exudate of red cells and polymorphonuclear leukocytes. The pleura of the right lower lobe showed a dense fibrinopurulent exudate in which Gram-positive cocci were found. The vegetations on the heart were rheumatic and were not the result of the pneumococcus infection.

This case illustrates the entrance of organisms into the lungs following an acute inflammation of the nose and throat. There was subsequently a direct spread to the pleural cavity. There was at first an absence of leukocytosis, then a leukopenia, and finally, after the administration of specific serum, a marked leukocytosis. The leukocytes at first were either inhibited or destroyed in the early stages of the infection, probably as a result of the action of the organisms. Following the administration of serum, this effect was in some way altered. After apparent improvement and after the blood had become free from organisms, the heart failed, probably as a result of an old rheumatic lesion (Fig. 2).

III. THE ALIMENTARY CANAL

In the mouth, the conditions are somewhat different from those in the nose. The mucous membrane of the oral cavity is firmer, and the glands of the mucosa do not respond by secretion as actively to mechanical stimuli on the surfaces. Around the teeth, however, there are innumerable crevices into which the bacteria may be carried, along with small particles of food, where they may grow in such a manner as to injure the mucous membrane. Any injury to the mucous membrane around the teeth, either mechanical or chemical, permits the organisms to penetrate and causes an exudation of cells and fluid from the injured surface. In such manner, also, the crypts of the tonsils may incarcerate organisms that, because of their multiplication and inability to discharge on the surface, may injure the mucous membrane and penetrate into the deeper tissues, as illustrated by Cases II and IV in Chapter XIII. Sharp objects, such as fishbones or shells, may actually perforate the mucous membrane of the mouth, throat, or esophagus, and carry with them the mouth microbes. Infections that follow are particularly severe because of the nature of the organisms introduced. In almost all cases, several species are found, none of which alone will produce similar lesions in experimental animals. It is therefore probable that the severity of the lesions is caused by some synergistic action of the bacteria. Operations in these regions are fraught with grave danger of infection, as Cases VI and X illustrate.



FIG. 4. CASE VI: Abscess of lung, × 15. *Insert* Mixture of organisms in lung abscess, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

tions to 40. Pneumococcus Type IV was recovered from the sputum. The temperature came down a little during the next few days; but on the fifth day rose again to 104°, where it remained until death on the eighth day after operation. Also the lung involvement progressed until both lobes became full of patchy areas On the eighth day, the patient coughed up some foul necrotic material. He became cyanotic and died with respiratory failure.

AUTOPSY. There were extensive sloughing and cellulitis of the tissues of the floor of the mouth and neck but no venous thrombosis was found. The pericardium and heart were normal. A small quantity of free turbid fluid was present in the right pleural cavity and some fibrinopurulent exudate over the lower lobe. There was a localized pocket of pus between the left lung and the diaphragm. The right lower lobe showed many dense irregular areas of consolidation, many of which had central necrosis, whereas the upper and middle lobes contained a few areas of consolidation. The right bronchus throughout its extent showed acute inflammation of the mucous membrane and contained pus. The left lung showed a similar condition of less extent.

Microscopic examination revealed edema and cellulitis of the muscles of the floor of the mouth, with bacteria present in the tissues. The lung abscesses contained mixtures of organisms. Culture from both lungs showed at least four different types of bacteria, including pneumococcus Type IV.

This case illustrates a postoperative pneumonia following an operation on the mouth. When recovering from anesthesia, the patient's throat was clogged with sticky mucus, and then coarse râles developed in the bronchi, followed by lung consolidation. In this case, the evidence seems to be in favor of an entrance of organisms into the upper respiratory tract and a direct spread downward to the lungs, gangrene developing in the presence of a mixture of organisms. Mouth bacteria also invaded the tissues of the neck locally, but there was no evidence of spread by way of big venous thromboses. Microscopic thromboses were not found but could not be absolutely ruled out (Figs. 3, 4).

Likewise, severe infection may follow human bites, for mouth organisms are introduced deeply into the tissues. That similar infections do not take place when wounds are sucked is probably due to the limited depth of introduction of organisms. There is some doubt with regard to the ability of certain particularly virulent organisms to penetrate a normal mucous membrane. Some consider this impossible even with the most virulent, whereas others believe that certain types may pass through intact mucosa. The consensus seems to be that the diphtheria bacillus and certain strains of hemolytic streptococci may pass through the intact mucous membrane of the nose and throat. Unquestionably, frequent cleansing of the mouth and brushing of the teeth remove countless organisms. Bacteria, however, are invariably present in the mouth, and when food is swallowed, not only the organisms that are contained in it, but the mouth organisms as well, are carried down to the stomach in large numbers. The destruction of bacteria in the stomach will be considered in Chapter xv.

It is certain that a portion of these ingested organisms, even under normal conditions, pass over into the duodenum. Numerous studies of the flora of the intestinal tract indicate that the bacteria increase enormously in numbers down to the ileocecal valve.[5] They die off to some degree in the colon. The bacterial content of the feces has been estimated roughly as one-third of the mass, although a large proportion of these organisms are not viable when the feces are discharged.[6]

With the food there is always present a certain amount of indigestible material. This is present to a considerable degree in certain vegetables and in the seeds of fruit. At times, hard particles are ingested, which may cause mechanical irritation of the mucosa. In certain places, as for instance the appendix, the fecal mass may become dry and hard and then produce a mechanical irritation of the mucous membrane, which may render it penetrable by the micro-organisms that are present. In the surgical pathology laboratory of the Presbyterian Hospital, New York City, Stout has been able to demonstrate a break in the mucosa in almost every case of acute appendicitis (Fig. 5).



FIG 4 CASE VI: Abscess of lung, × 15 *Insert* Mixture of organisms in lung abscess, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

tions to 40. Pneumococcus Type IV was recovered from the sputum. The temperature came down a little during the next few days; but on the fifth day rose again to 104°, where it remained until death on the eighth day after operation. Also the lung involvement progressed until both lobes became full of patchy areas. On the eighth day, the patient coughed up some foul necrotic material. He became cyanotic and died with respiratory failure.

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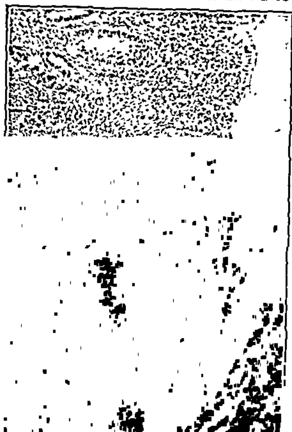


FIG 5. Appendix showing a break in the mucosa found after several examinations only at the tip, in a case of acute appendicitis, × 100 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

Under normal circumstances, the degree to which the organisms of the intestine pass into and through the mucous membrane of the gut is difficult to demonstrate, but certain organisms unquestionably enter the mucous membrane and penetrate into the walls of the intestine, while others may enter the lymphatics or the portal venous radicles. It may be that in every such case there is first some mechanical injury. It is generally believed, however, that the typhoid, dysentery, and cholera organisms may pass through an intact intestinal mucosa.[7] Bartel[8] states that the tubercle bacillus will also penetrate the intact gut. Particularly in the lower intestine, certain organisms find in the partially digested food the necessary requirements for their growth and multiplication. In this development, they produce certain metabolic products that may injure the membrane to such an extent that the organisms may penetrate the deeper tissues. Such may be the preliminary action of the organisms of typhoid fever, cholera, dysentery, and tuberculosis. Any injury to the intestinal mucous membrane, whether mechanical or chemical or the result of ulceration, either benign or malignant, opens the door not only to the organisms that may be directly responsible for this injury but to any other organisms present in the gut. This point is well illustrated by Cases VII and VIII in this chapter and Case III in Chapter XIII.

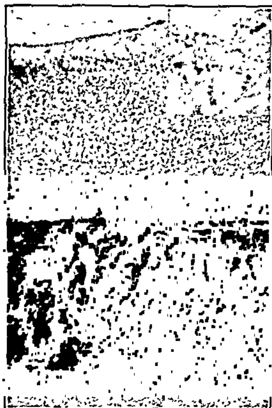


FIG 6 CASE VII. Nonhemolytic streptococcus peritonitis following perforation of duodenal ulcer Thick fibrinous exudate on the surface of the intestine Extreme injection of the blood vessels of the serosa, × 50 *Insert* Streptococci in peritoneal exudate, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

Case VII Name W.M. Age 52. History no 61970 Autopsy no. 9604.

HISTORY. The week before admission, the patient began to have indefinite lower-abdominal pains, chiefly on the right side The pain continued intermittently until two days before admission, when the man was suddenly seized with a pain much more severe, but still in the right lower abdomen. This was accompanied by repeated vomiting, without relief, until he had been given a large dose of morphine.

PHYSICAL EXAMINATION This showed an extremely dehydrated, prostrated man, apathetic and confused. The abdomen was flat and rigid throughout. It moved very little with respiration It was tender all over, but the maximum tenderness was on the right side of the umbilicus The liver dullness was not diminished. The white blood count was 10,000, with polymorphonuclears 74 per cent. Temperature on admission was *101.4° F*, pulse *120*, and respirations 24.

COURSE. Immediate operation was performed, free air and about 2000 mils of thin, yellow fluid containing flakes of fibrin were present in the abdominal cavity, with a perforated ulcer on the anterior surface of the duodenum. The opening was 2 mm. in diameter in the center of an indurated mass approximately 3 x 3 cm The perforation was closed and a jejunostomy done. Culture of the pus from the peritoneal cavity revealed nonhemolytic streptococcus. On the seventh day after operation, there was a disruption of the abdominal wound with a protrusion of the viscera This was closed, and for three days

after the second operation the patient held his own, but on the fourth day his temperature began to mount, his respirations became rapid, and he developed signs of pneumonia. His blood pressure fell, and he died on the fifth day after the second operation.

AUTOPSY. The abdominal cavity contained a small amount of fluid, the peritoneum was greatly injected, and there was a walled-off abscess under the diaphragm at the lower border of the anterior surface of the liver, containing about 500 mils of pus. The stomach was greatly dilated, and there was a fibrinopurulent exudate over the anterior surface extending on to the duodenum. A perforated ulcer was found in the duodenum almost a centimeter in diameter, covered by fibrinous adhesions. On the opposite surface of the duodenum, another ulcer was found, penetrating through the mucous membrane. The lungs showed a diffuse bronchopneumonia. Microscopic examination of the peritoneum showed a thin, fibrinous exudate containing large numbers of Gram-positive cocci.

This case illustrates the entrance of bacteria into the peritoneal cavity from a perforated ulcer of the duodenum. The organisms were moderately virulent nonhemolytic streptococci, found mostly in the duodenum They had spread extensively in the peritoneal cavity and had produced a large subdiaphragmatic abscess (Fig. 6).

Case VIII. Name A.S. Age 46. History no. 61398. Autopsy no. 9573.

HISTORY. Three days before admission, the patient began to have abdominal cramps. At first, they spread across the lower abdomen, but later became general. Soon after the pain began, he took some medicine and then vomited a large quantity of food. The pain subsided somewhat during the next day, but vomiting continued.

PHYSICAL EXAMINATION. The patient did not appear markedly toxic nor prostrated. The chest was negative The abdomen was markedly distended but without spasm There was general, moderate tenderness all over the lower half, more marked on the right side near McBurney's point, where, on deep pressure, a small, nodular mass could be felt. There was rectal tenderness high up on the right side. The white blood count on admission was 24,200, with polymorphonuclears 96 per cent. The urine showed a very faint trace of albumin and a trace of bile. Temperature on admission was 100.8° F., pulse 114, respirations 22.

COURSE. The patient was operated on at once with a lower right rectus incision. On the right side of the pelvis, extending up to the cecum, was a small abscess, containing thick, foul-smelling pus. A moderate amount of seropurulent fluid was also present in the pelvis and right lumbar gutter. The appendix was entirely gangrenous, the small intestine was considerably dilated The appendix was ligated and removed. The pelvis was drained through a small, lateral drainage opening. The right rectus wound was closed, and a jejunostomy made high up under the left costal margin. Next day, the patient was the picture of peritonitis with paralytic ileus. Foul, gaseous, brown fluid



Fig 7. Case VIII. Gangrene of the appendix with gas gangrene of the abdominal wall Gangrenous appendix, × 12. (*From Nelson's Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig 8 Case VIII: Peritoneal exudate on large gut, × 15 *Insert* Mixture of bacteria in peritoneal exudate, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

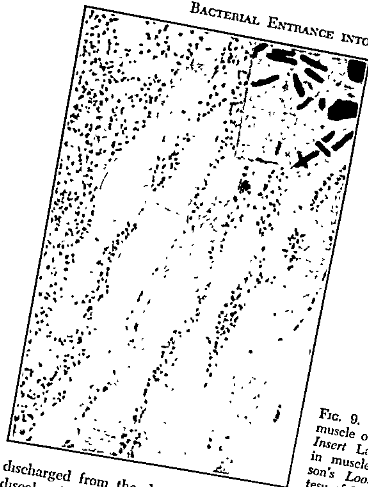


FIG. 9. CASE VIII: Disintegrated muscle of abdominal wall, × 100 Insert Large Gram-positive bacilli in muscle, × 1600. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

discharged from the drainage tracts. The margin of the rectus wound was discolored and swollen. No crepitation was made out, but when the wound was opened, bubbles of gas appeared. Cultures from the wound showed hemolytic *Clostridium welchii*, nonhemolytic streptococcus, *Escherichia coli*, and *Staphylococcus albus* The blood urea was 0 58 gram per liter. On the third morning, the patient began to fail rapidly. Jaundice appeared. The wounds were badly infected but without crepitation. A specimen of necrotic tissue cultured *E. coli*, nonhemolytic streptococcus, *C welchii*, and *C. sporogenes*. The temperature gradually rose to 105° F., and pulse to 140. The blood pressure fell to 78, and death occurred on the third day after operation.

AUTOPSY There was general jaundice The abdomen was somewhat distended There was an intense, greenish discoloration over the left half of the abdomen, beginning at the margins of the surgical incision just to the right of the midline and extending from the umbilicus to the symphysis and over the crest of the ilium up to the lower border of the ribs. Distinct crepitation was present. When the rectus muscle was cut, a thick fluid oozed out, through which bubbles of gas arose. When the sheath was opened, dark red, foul-smelling material of about the consistency of porridge flowed out. There was no excess of fluid in the peritoneal cavity. In the region of the incision, the intestine was lightly glued to the parietal peritoneum by a fibrinopurulent exudate. In the pelvis, there was a loop of intestine bent upon itself and

obstructed. Above this point, the gut was dilated and below, it was collapsed. Microscopically, the peritoneal exudate showed a mixture of intestinal organisms. The rectus muscle was fragmented and necrotic and infiltrated with large Gram-positive bacilli.

This case illustrates the entrance of bacteria into the physiological interior of the body through a perforation of the appendix. Then followed a direct spread from the peritoneum to the tissues of the abdominal wall, where the mixture of organisms including *C. welchii* produced clinical gas gangrene. Note that there were at least two anaerobic and three aerobic species of bacteria found in the infectious process (Figs. 7-9).

When Peyer's patches become infected in typhoid fever and the overlying mucous membrane becomes ulcerated, a wide door is opened for the entrance of all of the intestinal organisms. The surprising thing is that more of them do not become active in the production of a virulent infection. In like manner, the primary lesion of tuberculosis probably occurs in the deeper layers of the gut wall before the ulceration of the mucosa takes place. However that may be, the penetration of these organisms from the lumen into and through the mucous membrane presupposes a pathway, however small.

Any obstruction to the onward movement of the intestinal contents increases the possibility of mechanical and chemical injury because of the greater time of contact of the irritating substances and because of the pressure exerted upon the gut by the accumulated intestinal contents or gases. This may result in a cutting off of the blood supply so that the surface cells may actually die and disintegrate or be torn apart, thus opening up large pathways for the entrance of organisms.

The degree to which bacteria pass up the bile and pancreatic ducts has never been satisfactorily determined.[9] Bacteria are found in the gall bladder, both with and without stones. Certainly stones exist without cultivable bacteria, and in some cases of gallstones, the presence of bacteria may well be secondary. Not infrequently, large stones ulcerate through the walls of the gall bladder and intestine and produce a fistula that permits the direct passage of organisms from the gut into the biliary tract. This condition is illustrated by Case III in Chapter XIII.

IV. THE GENITOURINARY TRACT

As has been stated above, organisms are not normally present in the male genitourinary tract, except near the orifice. The frequent passage of urine tends to wash out this channel. During intercourse, the congestion of all of the tissues, including the mucosa, renders it soft and produces a favorable condition for the invasion of specific organisms,

provided they are introduced. It would seem likely that the penetra of the gonococci into the membrane of the male urethra corresponds some degree to the entrance of the nasal organisms into the congest membrane of that region. After the sexual act, the urethra collapse and crypts and folds are produced in which organisms may be retained Their development may be favored by the secretions, and the metabolic products of their growth are probably the cause of the irritation permitting their penetration, for it is not often that there is any mechanical injury to the urethra. This, of course, does take place on the outside of the penis, and it is believed that the entrance of spirochetes may depend upon a break in the continuity of the surface of the mucous membrane of the glans or foreskin.[10]

In the female, the separate orifices of the genital and urinary tracts permit quite independent infections to develop in the two tracts. A short passage from the bladder to the outside subjects the urinary tract more easily to the entrance of the organisms constantly bathing its orifice, namely those that come from the vagina and from the anus, particularly in children, where the control of the anal orifice is not as efficient as in adults and where the distance is shorter. The mucous membrane of the vagina is flatter and firmer than the urethra, but when empty it possesses folds and crypts in which organisms and secretions may be retained. Gonococci can live in the secretions of the cervix uteri and the vagina and can withstand the action of the normal flora.[11] By their irritating metabolic substances, they may cause injury permitting their penetration into and through the mucous membrane. The glands of the vagina and the cervix of the uterus are more vulnerable than the vagina itself. After inflammation has become established, it may extend either on the surface or beneath the surface more deeply into the genital tract. When instruments are passed into the uterus, some bacteria are usually carried in, because of the difficulty of local sterilization. Particularly in the presence of altered physiological conditions, as following pregnancy, the bacteria may find conditions favorable for growth. Case IX illustrates this point.

Case IX. Name A S. Age 40. History no. 51034. Autopsy no. 9147.

HISTORY. Five days before admission, the patient was seized with a severe abdominal pain, centered at first around the umbilicus and spreading later to the upper abdomen and right side. It steadily increased in severity. Vomiting began early and persisted until the time of admission. Prostration was profound and fever high. The patient had had nine pregnancies to term and many miscarriages. She denied pregnancy or miscarriage in the present illness, but after death, a relative stated that she was known to have been pregnant.

PHYSICAL EXAMINATION The patient was a very obese woman, evidently desperately ill. She was cyanotic. Her breathing was rapid and shallow, and her skin was cold and moist. The pulse could not be felt; the heart rate was



FIG. 10 CASE IX: Hemolytic streptococcus peritonitis following criminal abortion Purulent exudate in cavity of uterus, × 50 *Insert* Streptococci in uterus, × 1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

160. The abdomen was tender all over, with the most acute area around the umbilicus, and rebound tenderness over all There was bleeding from the vagina The cervix was soft and patulous, with a whitish discharge The temperature was 105° F., and respirations 40 The white blood count was 22,000, with polymorphonuclears 88 per cent

COURSE. Immediate operation revealed a general peritonitis with thin, yellowish, odorless pus, without any definite focus being found. Immediately after the operation, the patient became very much more cyanotic and died ten minutes later. Culture of pus from peritoneal fluid showed a hemolytic streptococcus and *Bacillus subtilis*

AUTOPSY. The thoracic organs were normal except for many small hemorrhagic areas in both lungs. The peritoneum contained a moderate amount of free pus, very much more in the pelvis than elsewhere in the abdominal cavity The tubes were dark red in color, and both they and the uterus contained a thick, yellow pus. The mucous membrane was greatly injected, and there were large areas on the inside of the uterus where the surface was rough, dark red in color, and necrotic. The cervix was large, and its canal readily admitted the passage of a finger. The other organs were relatively normal except for a diffuse enlargement of the spleen Microscopic examination showed evidences of early pregnancy in the uterus, tubes, and the left ovary. Gram-positive cocci in chains were found both within and without the uterus and tubes



FIG. 11. CASE IX: Intense congestion of the Fallopian tube with fibrinous exudate on the surface. (*From Nelson's Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)

This case illustrates the entrance of bacteria by way of the genital tract, following trauma, during an altered physiological state, and a direct spread through the uterus and tubes to the peritoneal cavity (Figs. 10 and 11).

In certain circumstances of obstruction of the urinary tract, as for instance by a stricture of the urethra, by hypertrophy of the prostate, or by spasm of the sphincter, not only may there be a mechanical injury to the membrane, which permits any organisms present to invade the tissue, but there may also be a chemical injury caused by the delay in the discharge of the irritating fluids. Organisms are almost invariably introduced into the bladder when instruments are passed, because of the difficulty of sterilizing the orifice; and these, either by their specific irritating action or by taking advantage of any other mechanical injury, may penetrate into the tissues. The consensus of genitourinary surgeons is that the organisms so introduced rarely, if ever, cause an infection unless there be an associated obstruction to the outflow of urine or the presence of residual urine.[12] Interesting lesions of this tract are illustrated by Cases XVII and XVIII in Chapter XIII.

V. THE CONJUNCTIVA

The air organisms are constantly coming in contact with the conjunctivae. The dust is usually quickly removed by the act of winking and the flow of lacrimal secretion. Not infrequently, however, particles injure the surface epithelium and, by a break in the continuity, permit the entrance of organisms. Certain organisms, such as staphylococci and gonococci, have a greater tendency than others to produce an inflammation of the conjunctivae, either because of an irritating action of their own or by entering through an injured surface. It is certain that relatively few species of organisms ever penetrate and produce inflammation in the conjunctiva, although countless organisms are constantly present on its surface. Bacteria that are washed down through the duct occasionally delay long enough to grow and produce a local infection in the lacrimal sac, or if a frank infection of the conjunctivae occurs, the organisms and pus draining through the lacrimal duct not infrequently produce an inflammation in the lacrimal sac.

VI. 'CRYPTOGENIC' INFECTIONS

When bacteria enter the body, a reaction usually takes place that indicates the point of entrance. Frequently, however, the first evidence of inflammation is in the physiological interior of the body, and we assume that the organisms have passed through the surface wall without such local reaction. In these cases, the entrance point may be on one of the internal surfaces, and the reaction there may not cause subjective symptoms. Such for example, are Cases x and xi below, and Case v in Chapter xiii.

Case X. Name M. R. Age 58. History no 63118. Autopsy no. 9659

HISTORY. The patient had suffered from vague abdominal pain off and on for a year. This became more pronounced two months before admission and was accompanied by vomiting and nausea, which had no relation to meals At the same time, she began to have chills, which came nearly every day and lasted several hours. They continued for four or five weeks Five weeks before admission, she began to have pain in the lower back She was confined to bed Two weeks before admission, she began to feel a hard lump in her rectum, and the pain in the back increased in severity. She had lost nearly forty pounds in two months.

PHYSICAL EXAMINATION The patient was a well-developed, elderly woman, appearing chronically ill. Her skin was dry and wrinkled The head and chest were relatively normal. The abdomen was distended and slightly but generally tender. A large mass was felt in the rectum, which was thought to be inspissated feces. Tenderness was present over the lower lumbar vertebra, over the



FIG. 12. CASE X: Cryptogenic nonhemolytic streptococcus septicemia. Wall of abscess of pelvis, × 12. *Insert* Streptococci in abscess, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



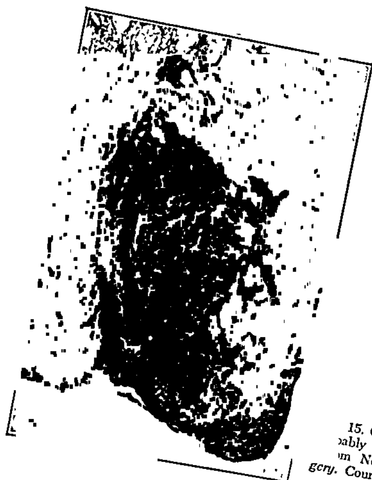
FIG. 13. CASE X: Thrombosis of inferior vena cava, × 12 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 14. CASE X: Bacterial masses in thrombus of inferior vena cava, × 150. *Insert.* The same, × 1500. (Photographs by Dr. W. C. von Glahn From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

sacrum, and over the sacroiliac joints. There was also tenderness over both lower extremities on deep pressure, with slight edema of the ankles. The blood count showed red cells to be 3,100,000, with hemoglobin 50 per cent; the white cells were 18,600, with polymorphonuclears 76 per cent. The stool was positive for blood. On admission, the temperature was 99 4° F., the pulse rate 104, and respirations 26.

COURSE The patient's temperature varied for the first week between 99° and 102° F., but rose to higher levels, from 102 to 103 8° F., in the second week. On the eighth day, she developed an acute parotitis on the left side. Blood cultures were thrice negative during the first three weeks. None was taken during the later stages Cultures taken from the parotid duct opening showed hemolytic *Staphylococcus aureus* and a nonhemolytic streptococcus. With local applications, this quieted down for a time, but during the fourth week, it recurred, and with it the temperature rose to 104 4° F., and the patient became irrational. The white blood cells rose to 44,600, with polymorphonuclears 92 per cent. The right leg then began to swell, and an x ray showed an extensive osteomyelitis, but there was very little reaction of the tissues. The patient gradually became moribund during the fifth week The temperature fell to normal, but the white blood count remained high For two weeks she lay unconscious and could not be aroused. She refused to take nourishment by mouth and died seven weeks after admission.



15. CASE X: Abscess of lung, ›ably a 'septic infarct,' × 15 ›m Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

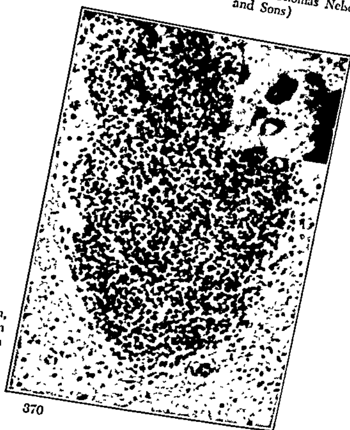


FIG 16. CASE X. Abscess of brain, × 200 *Insert*. Streptococci in brain abscess, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 17. CASE XI: Cryptogenic hemolytic *Staphylococcus aureus* septicemia. Abscess of epididymis, × 15. *Insert.* Staphylococci in epididymis, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 18. CASE XI. Thrombosis of pelvic veins with masses of bacteria on the surface, × 200. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

AUTOPSY. The body was that of a fairly well-developed, but emaciated, middle-aged woman with bedsores on the sacrum and on the heels. The left parotid gland was swollen and contained pus. In the thoracic cavity, both lungs were attached to the parietal pleura by loose, fibrous adhesions. The heart was relatively normal. In the upper lobe of the right lung, a cavity was found containing pus. In the left lung were a number of small abscesses. The peritoneal cavity appeared normal above the pelvis. The liver, spleen, and kidneys were relatively normal. The gastrointestinal tract was negative, except that the colonic mucous membrane had a brown discoloration. No ulcers were found. The pelvic organs were negative, but on the left side of the pelvis, a firm mass was found between the uterus and rectum with many small pockets containing greenish exudate. The inferior vena cava showed a brownish green thrombus practically filling the lower 10 centimeters. In the lower part of this and passing down both common iliac veins, this thrombus was partially liquefied and contained bright green pus, on the right side extending down into Hunter's canal. A large abscess was found in the right psoas muscle. The brain and cord were grossly normal. Both femurs showed a reddened bone marrow without pus. Culture from the heart yielded a nonhemolytic streptococcus; from the psoas muscle the culture was overgrown with *Bacillus proteus*.

Microscopic examination showed the heart to be normal. The lungs showed many small abscesses filled with polymorphonuclear leukocytes. The spleen, liver, kidneys, and pancreas were relatively normal. The pelvic mass showed scar tissue and granulation tissue lining an abscess cavity, in which were many large thrombosed veins. The thrombi were partially organized. The inferior vena cava showed a suppurative thrombus also partially organized. In the pelvic abscess wall, in the vena cava and femoral veins, Gram-positive cocci were seen. The left parotid gland showed multiple abscesses. The brain revealed a number of microscopic abscesses and thrombosed vessels. Gram-positive cocci were found in the parotid gland and brain, and in the smears from the marrow from the right femur and third lumbar vertebra.

This case illustrates a cryptogenic infection with a nonhemolytic streptococcus, possibly arising from the lower gut, inasmuch as the pelvic abscess seemed to be a very old process. The iliac veins and vena cava became thrombosed, and the bacteria spread by the venous system to the lungs and thence to the arterial side, forming abscesses in the brain and bone. The parotid gland may have had a primary or secondary infection from the mouth by *Staphylococcus aureus* (Figs. 12-16).

The frequency of the staphylococcus as the causative organism in many of these 'cryptogenic infections' suggests that they may gain entrance through trivial openings in the skin. Such infections are particularly common in children. Case XI illustrates this type of infection. The reason for the absence of reaction in such cases will be discussed in Chapter XII.

Case XI. Name R.W. Age 14. History no. 55071. Autopsy no. 9291.

HISTORY. One month before admission, the patient had a series of boils on the left wrist, which were lanced, and healed in two weeks. During this period, he had no fever and did not feel ill. Three days before admission, the patient ate frankfurters and drank lemonade. That night, he felt feverish and complained of abdominal pain. On the next morning, he had a chill followed by fever of 103° F. He then stated that the pain was most intense in the left inguinal region, which was tender. All during his illness, he had been very drowsy following the chills, and his fever recurred. The temperature reached 106° F.

PHYSICAL EXAMINATION. The boy appeared to be acutely ill. His face was flushed, and he was drowsy. The skin was dry. On the left wrist there was a small scar, the remains of a small boil There was no tenderness, lymphangitis, or lymphadenitis in the neighboring parts. The throat was diffusely red. Nothing abnormal was found in the lungs nor in the heart. The heart action was rapid and the pulse dicrotic. The abdomen was greatly distended, but not tender, except in the left inguinal region The spleen was readily palpable. The white blood count was 13,000 and polymorphonuclears 80 per cent. The temperature on admission was 105° F., the pulse 100, and the respirations 36.

COURSE. During the first night, the patient had a severe nose bleed and later became wildly delirious. A typhoid agglutination test was negative. Blood culture revealed hemolytic *Staphylococcus aureus* When plated with agar, the blood showed 150 colonies per cubic centimeter. On the second day, the white blood count rose to 25,000 with polymorphonuclears 77 per cent. The tenderness in the left inguinal region became more marked On the third day, a small pustule appeared in the region of the previous boils. This yielded hemolytic *Staphylococcus aureus.* A systolic murmur developed in the heart. On the fourth day, all movements of the legs became painful, and the left ankle became swollen. Pus was aspirated from the ankle joint, and the joint was opened. On the fifth day, an abscess developed in the right sternomastoid region, and subsequently, a number of scattered superficial abscesses appeared. These were opened. On the seventh day, pus was found in the urine. On the tenth day, signs of bronchopneumonia appeared in both lungs with overlying friction signs. The temperature continued at a high level, between 102° and 105° F. The patient became increasingly stuporous, with signs of meningeal involvement, and finally died on the seventeenth day after his admission.

AUTOPSY Externally, a number of draining surgical wounds were present The left epididymis was greatly swollen. The peritoneum was normal, but in the pelvis was a soft, fluctuating abscess on the left side, which was retroperitoneal. The abscess filled the left half of the pelvis and extended upward beneath the iliacus muscle to the crest of the ileum and beneath Poupart's ligament into the thigh It contained about 300 mils of thick, yellow pus. The left common iliac vein and its branches in the neighborhood contained soft thrombi A large abscess was found in the left epididymis The prostate was normal In the heart, a mural thrombus was found in the left ventricle, and

several small abscesses were found in both lungs, but no thrombi were present in the pulmonary arteries. The surfaces of both lungs were covered with a fibrinopurulent exudate, with several small pockets in the pleurae. Septic infarcts were found in the spleen and kidneys. Several small abscesses were found in the wall of the intestine, and many small abscesses in the liver. The microscopic examination showed abscesses in all parts of the body to contain clumps of Gram-positive cocci. Cocci were also found in the infected thrombi in the pelvic veins.



FIG. 19. CASE XI: Thrombus on inner surface of left ventricle; x = mural thrombus (Photograph by Dr. W. C. von Glahn. From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



Fig. 20 Case XI· Abscesses in kidney (Photograph by Dr W. C von Glahn. From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



Fig 21 Case XI: Subpleural abscess of lung, × 15 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

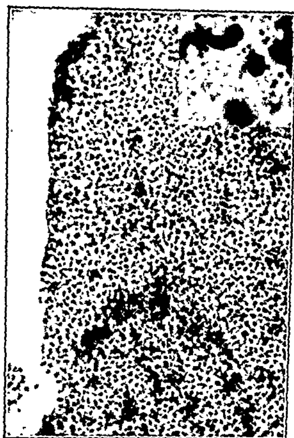


FIG. 22 CASE XI: Cellular infiltration of thrombus on heart wall, × 200. *Insert:* Staphylococci in thrombus, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 23. CASE XI: Abscess of spleen, × 15 *Insert* Staphylococci in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig. 24 Case XI. Metastatic abscesses in kidney, showing bacterial mass in abscess, × 200. Both glomeruli and tubercles are involved. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig. 25. Case XI Metastatic abscesses in intestinal villus, × 12 Note the bacterial masses in the center of each of the three abscesses, × 20 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

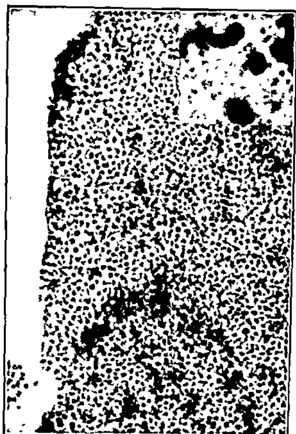


Fig 22 Case XI. Cellular infiltration of thrombus on heart wall, × 200. *Insert:* Staphylococci in thrombus, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig 23 Case XI. Abscess of spleen, × 15. *Insert.* Staphylococci in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 24. CASE XI: Metastatic abscesses in kidney, showing bacterial mass in abscess, × 200. Both glomeruli and tubercles are involved. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 25. CASE XI: Metastatic abscesses in intestinal villus, × 12. Note the bacterial masses in the center of each of the three abscesses, × 20. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 26. CASE XI: *Abscess of spleen. (Photograph by Dr. W. C. von Glahn. From Nelson's Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)

This case illustrates a cryptogenic infection in a boy of 14. The organisms may have made their original entrance several weeks preceding the present illness and localized in some internal focus, lying dormant there for a time but subsequently developing locally and spreading. The evidence seems to be that this focus was in or near the pelvis, possibly in the epididymis. The iliac veins then became thrombosed and infected. Subsequently, the organisms spread by both venous and arterial circulation to the lungs and other parts of the body (Figs. 17-26).

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CHAPTER XII

Bacterial Establishment within the Human Body

THE INVASION of dead tissue by bacteria is one of the most important processes of Nature, for by it the tissue is reduced to those elements that in the cycle of life are necessary for the synthesis of new plants.[1] On the other hand, in Zinsser's opinion,[2]

> . . . true infection is a process quite out of keeping with the ordinary plans of Nature. . . There seems to be a distinct opposition to the colonization and functionation of one living being within the living substance of another. . . It would seem to rest in principle upon the fact that the invading cell meets the invaded one under conditions peculiarly adapted to the activities of the latter and is overcome before conditions suitable for its own activities have been established.

However, both in plant and animal life, there are exceptions to this rule, and it is found that certain bacteria can grow and multiply only in the tissues of another living organism. 'Many pathogenic organisms' that undoubtedly grow in the animal body 'have so far baffled all of the efforts that have been made to cultivate them in artificial media.'[3] We have the parasitic plants, the mistletoe and orchid, which will not grow alone. Likewise, there are bacterial infections, such as gonorrhea and syphilis, in which the perpetuation of the bacterial species depends upon direct transfer from one individual to another; and although the organisms may exist for a certain length of time outside the animal body, under natural conditions they do not multiply there but readily perish. There are, also, those micro-organisms that have an alternating life cycle in two different hosts, such as certain of the protozoa and the helminths, and the still more interesting 'rust' parasite of the white pine and gooseberry, which alternates from season to season in the selection of its host.[4] When we consider all of the parasitic organisms of the lower animals and plants, we find that there exists, after all, a large group of living beings that, in the present state of evolution, must invade the tissues of other living beings if they are to continue perpetuating their kind.

I. BACTERIAL EVOLUTION

When one considers the phase of evolution in which the parasitic organisms first appeared, the following questions arise: (1) Did they exist before the hosts that they now require for their existence? (2) If so, in what form did they exist? (3) How did they change their life requirements? Osborn[5] believes that bacteria are primordial forms of life and that their parasitic characteristics have been secondarily acquired. He says:

> A bacterialess earth and a bacterialess ocean would soon be uninhabitable either for plants or animals, conversely it is probable that bacteria-like organisms prepared both the earth and the ocean for the further evolution of plants and animals and that life passed through a very long bacterial stage. . . In their power of finding energy or food in a lifeless world the bacteria known as prototrophic or primitive feeders are not only the simplest known organisms but it is probable that they represent the survival of a primordial stage of life chemistry. . . It seems that the early course of bacterial evolution was in the line of developing a variety of complex molecules for performing a number of metabolic functions and that the visible cell differentiation came later. . . The parasitic life of bacteria beginning with their symbiotic relations with other bacteria was extended into intimate relations with the plants and finally with the entire living world

D'Herelle[6] has said that although we do not know the nature of the living state, we do know that the 'two fundamental attributes of living organisms are assimilation and adaptation. With bacteria, assimilation involves the faculty of multiplication When they grow to a certain size, they *must* divide, because surface increases as the cube of any dimension.' Assimilation can continue only by division of the cell when it has reached the point where the surface cannot provide it with sufficient nourishment Furthermore, the organism is dependent upon its surroundings for the elements required for its growth and it must be able to assimilate these elements from its environment. Inasmuch as its environment is constantly changing, it must be able to respond to these variations.

Any living organism, in order to grow and multiply, must have in its environment not only the elements necessary to this growth, but must also be able to initiate and carry on the processes that can utilize those elements in building up its specific body substances. If those elements are not present in a combinable form there must be another mechanism by which the more complex substances containing them can first be broken down We must assume, therefore, that bacteria, which are known to multiply enormously within the body tissues, must have these prop-

erties, and that the environment contains the elements necessary for their growth.

II. CHANGING ENVIRONMENT

D'Herelle has called attention to the fact that living organisms are constantly moving about among one another. For that reason, they are constantly coming into contact with a changing environment, which may be inert, or else biologically or chemically active. If these variations are too sudden or too intense for the organism to adapt itself to them, it must perish. A long series of successive adaptations undergone by a given individual differs somewhat from the series of any other individual. Organisms living for just a short period of time in any generation obviously cannot have made a great number of adaptations; for instance, bacteria in a single culture are all very much alike, particularly if they have all grown from a single organism or a small inoculum. More complex animals, such as mammals, will vary from one another infinitely more, because of their longer life and the infinite number of variations of their environment Laboratory animals, however, of the same litter and of approximately the same weight will respond in a fairly uniform manner to a given change in environment, as for example, the injection of bacteria; older animals of different litters and of different ages and weights may show marked differences. It is obvious that human beings will vary still more in their response to changes in environment—so much so that it is almost impossible to predict what the response will be to a given change, as, for instance, the entrance of bacteria into the body. However, in our experiments with animal inoculation and artificial media, we can control to some extent the environment of bacteria. By observing the reaction of bacteria under certain conditions, we can more readily predict what their reaction will be to a known change in environment.

It must not be forgotten, however, that experimental methods often do not simulate the natural processes of infection. This fact was emphasized by Welch [7] and was reiterated by d'Herelle. We inject into the body cavities or beneath the skin of normal animals large quantities of bacteria and their products in artificial media. We force these animals to inhale or aspirate countless numbers of organisms and attempt to draw conclusions from our observations when we know that in the production of natural disease, the dose must of necessity be very much smaller. Again, when we inject artificial cultures into animals, we know that we are subjecting the bacteria to profound changes in environment, whereas in natural infections the organisms are frequently transferred from the tissues of one animal to an almost identical environment in the tissues of another. Thus, when a direct transfer of organisms takes place, the conditions are most favorable for the development of the organism, and the

variation of environment is neither profound nor abrupt. At the same time, if the newly contaminated individual is normal, 'the invading organism will be meeting the tissues of the invaded one under conditions peculiarly adapted to the activities of the latter,'[2] and infection may not take place. If, on the other hand, the invaded tissue is abnormal, either because of local injury or general debility, or both, the change in the environment of the tissues may be both profound and abrupt; in this case, the local tissue cells may die, and subsequently the whole body perish

In natural as well as in experimental infection, organisms may have been subject previously to a different environment for an indefinite length of time. We must assume that when the contact of the bacteria with the tissues takes place, there is a biologic reaction between the two. Certain chemical processes are initiated either by the body or by the bacteria, or both.

III. BIOLOGIC ACTIVITIES OF HOST AND BACTERIUM SIMILAR

Let us assume for the moment that the bacteria can utilize the bodv fluids in the same way in which the body cells utilize them, namely, that they procure and take within themselves the oxygen and food elements, metabolize them in the same manner, and cast forth the same waste materials Even if the rate of growth differed considerably from that of the body cells, the surrounding cells would not be injured otherwise than by the growth of foreign cells. In that case, the local tissues and the body as a whole would not react to the bacterium any more than they would to the normally metabolizing body cell, and there would not be any evidence of disease, except, perhaps, an increase in the size of the part affected.

This apparently is true of some of the relations between certain bacteria and the animal body The tubercle bacillus, for instance, may live within the body of the white rat without producing disease, and may multiply moderately without disturbing the general condition of the animal and without poisoning its tissues[8] The same thing is true of certain of the protozoa, namely, trypanosomes in rats[9] and the Leishman-Donovan bodies of kala-azar in field mice.[10] An analogy may be drawn to a foreigner entering a country and living in exactly the same manner as the individuals who were already there He would be considered one of the inhabitants because there would be nothing peculiar about the way he lived or reproduced his kind. There would be no reaction against him; and, unless he prevented the life or multiplication of the inhabitants, he would be considered one of them.

IV. BIOLOGIC ACTIVITIES OF HOST AND BACTERIUM DISSIMILAR

If, however, the biologic activities of the bacterium and the host are different, the bacterium may not be able to utilize the body fluids in the same manner as do the body cells. In order to grow, it must modify these fluids in a different way or utilize some other element of the environment. In like manner, the waste products of the metabolic process may differ markedly from the waste products of the body cells and may have certain deleterious effects upon the tissues. These metabolic processes may be similar to the metabolism of the body cells or they may differ strikingly from them. The more they differ, the more they may interfere with the normal existence of the body tissues. The difference may be so slight that the organisms may grow for a long time without producing any reaction on the part of the body, as in certain chronic infections like tuberculosis. On the other hand, the difference may be so great that there is an immediate and violent reaction on the part of the body; such a condition is seen in acute processes like those caused by the hemolytic streptococcus. There is, of course, no certainty that the metabolic processes of bacteria, by differing markedly from the metabolic processes of the body cells, will be deleterious to the host other than by the mere loss of the food that the bacteria are utilizing. It is even conceivable that certain of the metabolic products of bacteria may be beneficial to the host, as is true of certain bacteria parasitic to the roots of leguminous plants It would be better for both the organisms and the host to grow together in a perfect symbiosis. The deleterious effects of the metabolic activities of the bacteria within the body may be a pure accident. For although it is conceivable that the best interests of the bacteria in the propagation of their race are served when the tissues of the host, at least in the immediate vicinity, are injured or destroyed, the purpose—if there be one—is thwarted if the host is destroyed, for then the bacteria in their turn must perish.

Organisms differing markedly in their metabolism from the cells of the host may be compared to foreigners who enter a country and do not conform in their growth and multiplication to the standards of the inhabitants. They require certain foods and special living conditions, and the by-products of these differences may react unfavorably upon the natives, by their multiplication, also, they may prevent the development and multiplication of the native population. Depending on the extent to which they differ, there is usually a reaction on the part of the group to which they are foreign, which either forces them to adapt themselves to the conditions of the natives, or forces them to withdraw from the com-

munity, or destroys them. Sometimes such foreigners may be segregated in certain localities, as in sections of a large city, where they can carry on their peculiar mode of life without greatly affecting the general life of the native inhabitants. In like manner, bacteria may be isolated in certain parts of the body, where they may carry on their peculiar metabolic functions without producing marked reactions by the body as a whole.

V. THE IMPORTANCE OF THE ROUTE OF ENTRANCE

The great majority of the bacteria, which we believe enter the tissues of the body when those tissues are open, do not find suitable environment for their development. They either perish or lie in a dormant condition, like seeds in unfavorable ground. Whether the pathogenic organisms when they enter the body tissues find favorable environment or not, depends upon the anatomical site of their entrance. Certain of the organisms find favorable environment only in certain tissues. For instance, cholera organisms find a favorable environment only in the intestinal tract. Other organisms, such as *Eberthella typhosa,* find favorable environment in several different tissues (for example, intestine, gall bladder, bone). Still other organisms, like the hemolytic streptococcus or *Staphylococcus aureus,* find a favorable environment in almost every tissue of the body. Whether the distinct tissue predilection of certain bacteria is due entirely to the presence of nutritive substances or to the absence of resistance of the tissues is as yet not clear. D'Herelle suggests that the affinity of certain tissues for certain bacteria is in the nature of a specific chemotaxis.[11]

VI. INCUBATION TIME IN BACTERIAL GROWTH

Since Barber's [12] first demonstration of the possibility of isolating single organisms, their growth requirements and growth phases have been better understood. When single organisms thus isolated are planted in culture media, many fail to grow. This does not mean that they are dead, but that they are not in the proper growth phase to take advantage of the environmental conditions. Burke, Sprague, and Barnes [13] have studied this question with *Escherichia coli.* They found that when single organisms were picked and replanted, 65 per cent began to multiply at once; 20 per cent more began to develop in the next twenty-four hours; 5 per cent more lay dormant for a longer period—some of them as long as sixteen days—before starting to grow Still others failed to grow at all. The same thing may be true in natural contamination of tissues. With the medium the same in all cases, how can it be that it is favorable for some indi-

viduals and not for others? The difference must be inherent in the individuals. Therefore, it is just as true to say that organisms are not favorable for the environment as to say that the environment is not favorable for the organisms. There must be some period in their life cycle in which they cannot utilize the elements in their environment or initiate those preliminary digestive processes that prepare the environment for assimilation. It is impossible to tell at just what age of the bacterium such conditions exist.

When bacteria are seeded into fresh-culture media, there is a preliminary period in which certain of them die off and the others fail to multiply. This is called a period of 'lag,' and corresponds to the period of adjustment to the new environment and a mobilization of the metabolic processes.[14-15] Then with increasing rapidity, the organisms begin to multiply until they enter the phase of most rapid growth. After a few hours, there is a gradual slowing up of the rate of multiplication as the products of the metabolism accumulate in the medium. Then the organisms begin to die off.

In like manner, both in natural and experimental disease, there is a period of incubation in which the patient experiences no symptoms and the animal shows no signs of the disease. This is conceived to represent two phases, the first being the period of lag in the bacterial growth or adjustment of the bacteria to their environment, and the second the time required for the action upon the body cells of the poisons that are produced. We know that this latter action takes time, because there is always some delay in the production of symptoms even following the injection of the most powerful toxins.

In artificial cultivation, the period of lag may be shortened by transferring the organisms during their most rapid growth phase. In like manner, the period of incubation may be shortened if organisms are transferred directly from one animal to another during the period of most rapid multiplication, as probably happens during an epidemic[14] among human beings.

It has been demonstrated by many observers that organisms grown upon an artificial medium previously enriched by the serum of a certain animal often become more virulent for that animal. The organism seems to adapt itself to the serum of the animal in the medium and to develop either some offensive or defensive mechanism that can be used in its favor when it comes into actual contact with the animal's tissues. Welch conceived the theory that bacteria may develop an immunity or a resistance against the tissues of the host or of the organ in which it has grown that makes it more adaptable to the environment and causes it to grow more readily if it is transplanted again to a similar environment.[17] This property is undoubtedly the basis of the tissue selectivity that certain

bacteria appear to possess. Even the organisms that are found commonly in many different kinds of tissue, such as streptococci, for a time apparently select for growth and multiplication certain tissues in preference to others.[18-20]

VII. THE METABOLISM OF BACTERIA

Bacteria are known to carry on their life processes by the utilization of their environment in a manner similar to that of other living things. The processes by which these changes take place as life goes on are generally summed up in the word 'metabolism.' Kendall[21] has given a great deal of consideration to these bacterial activities. A brief summary of one of his articles can best fulfil our purpose to give a concise idea of the general principles of this very complex subject.

The energy requirements of all living things depend largely upon the ratio of their surface to their volume, and the smaller an object is, the greater is the relative size of the surface to the volume. Thus, for their size, bacteria require a relatively enormous amount of nutrition; and inasmuch as they have no chlorophyll, the chief factor in the transformation of sunlight into growth in the more complex plants, they must depend upon the surrounding substances for their energy. The utilization of the food by the bacteria has two distinct phases, which may at times merge. The first is the constructive or anabolic phase, which is chiefly characterized by growth. The second is the energy or catabolic phase, in which the cell performs its peculiar and specific functions aside from growth. When bacterial bodies are analyzed chemically, it is found that they contain nitrogen, hydrogen, carbon, and oxygen, together with smaller amounts of phosphorus and inorganic salts, in very much the same proportions as do other plant and animal cells, except that phosphorus is disproportionately abundant. Nitrogen is the most essential vital structural chemical, and when 'in combination with hydrogen as amino nitrogen, it is the very cornerstone of life.'[22] Except for the nitrogen-fixing and nitrifying organisms, all bacteria require amino nitrogen for their metabolic activities. With some strains, this may be in very simple form; others require combinations similar to or identical with the nitrogenous compounds of the human or animal body.

The anabolic phase represents a weaving together of the necessary elements into the complex substance distinctive of the bacterial species In order that these elements may be available, it may be necessary for certain catabolic processes to act upon the surrounding medium. This may be done either by the cell itself or by its neighbors, and it is quite possible that for a certain stage in their life, organisms are dependent upon neighboring organisms for growth. This fact may explain why, as

mentioned above, single organisms when transplanted often fail to grow. Generally, large quantities of pneumococci and other organisms must be transplanted into the usual medium to produce any growth. In fact, certain organisms may have to die and disintegrate in order that others may have food upon which to grow.

The catabolic phase depends largely upon the character of the carbonaceous substances from which bacteria obtain their energy. The carbon is obtained chiefly from carbohydrates and proteins, and an organism in the presence of carbohydrate may produce substances quite different from those produced when it must utilize protein. This is true of many of the so-called putrefactive organisms. In general, carbohydrate will be utilized first if it is present, possibly because of its simpler chemical structure. The diphtheria bacillus produces its most potent toxin when protein and peptones are present. Similarly, the proteus bacillus produces a very active proteolytic enzyme when in contact with proteins, but grows readily on carbohydrates without the production of this substance Every organism acts characteristically and differently upon the common protein constituents of broth medium, so that the products are distinctive, whereas products of carbohydrate cleavage are often similar. For example, if protein or peptone is present in the medium, but carbohydrate is absent, the diphtheria bacillus and the Shiga dysentery bacillus produce their potent toxins and the colon bacillus produces indol; but when carbohydrate is present, all three produce lactic acid. Thus, in culture media, carbohydrate may spare protein just as it does in the metabolism within the human body.

Kendall[22] has made quantitative studies of nitrogen changes in standard culture media for many organisms, and the changes that take place from day to day indicate, more or less clearly, the process and the variations that take place as the products of metabolism accumulate. These, of course, differ from the changes that take place within the human body, first because of the difference in the material available for food and second because in the body many of the products of bacterial metabolism are constantly being swept away by the circulation. In general, the results of the earlier determinations are of more importance than those in the later stages of artificial cultivation. On the other hand, it has been demonstrated that the diphtheria bacillus produces the same toxin in the body as it does in protein-containing medium;[23] and after all, the specificity of the products depends rather upon the bacteria than upon the medium. Many bacteria, however, unquestionably produce their harmful effects only when they grow within the tissues of the body, for example, typhoid bacilli, nonhemolytic streptococci, and others.

The knowledge that carbohydrate spares protein in bacterial culture media has been utilized in the attempt to control the proteolytic activities

of bacteria within the intestinal tract. This has been attained to some degree by furnishing carbohydrate in sufficient quantities to prevent the production and absorption of toxic substances resulting from proteolytic activity.[24]

The metabolic activity of bacteria is accompanied by a liberation of energy, which usually takes the form of heat, but with certain bacteria is manifested by the phenomenon of light.[25]

Among the earliest functions of bacteria discovered was the fermentation of sugar, in the work of Schwann [26] and Pasteur.[27] Then followed the demonstration, by Pasteur, that putrefaction, which involved the splitting of fats and proteins, was carried on by bacteria [28] This catabolic action is carried out largely by enzymes, whose action takes place at some distance from the bacterium, on the surface of the bacterium, or within its body. Enzymes act as catalytic agents, hastening processes of decomposition without permanently entering into any chemical combination and without being used up in the process. The action of all enzymes is governed to some extent by environmental conditions The physical state, the temperature, and the reaction of the medium are all important. That bacteria have these enzymes has been demonstrated again and again [29, 30] In some cases, enzymes may be found in filtrates from living cultures, in which case it appears that the enzymes may be thrown off from the living cell and function at some distance from it Others are found only in old cultures when a certain amount of destruction of the organisms has taken place; it is thought that in the latter cases the ferments are held either on the surface or within the bodies of the organisms and are released only when they disintegrate.[31-2] Of similar nature are those enzymes that may be obtained only by extracting the organisms.

The difficulties of demonstrating the enzymes of bacteria lie in the fact that the optimum conditions must first be determined for temperature, medium, reaction, and so on. It is readily seen that unless the conditions of the body can be simulated, it is difficult to demonstrate the fermentative action of bacteria in the tissues. Clinically, there is much evidence in favor of the theory that certain organisms have ferments that directly attack the fat, protein, and carbohydrate of the body tissues. The most striking example of these fermentative actions is seen in the clinical infections caused by certain of the anaerobic organisms of the gas-gangrene group, which produce acid and gas in muscle tissue (Fig. 1).

The study of the chemistry of bacterial metabolism is in its formative state. The elucidation of many of the less obvious activities must await the development of new chemical methods.



FIG. 1. *Cross section* of *muscle* of abdominal wall *showing gas in the* muscle *fibers* and *extensive edema* with absence of cellular infiltration, × 200. *Insert*: Gram-positive bacilli in the *muscle*, × 1200. (Cultured *Vibrion septique*) (Bacteria photograph by Dr. W. C. von Glahn. From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

VIII. SPECIFIC BACTERIAL POISONS

These are truly products of bacterial metabolism, coming from the cell itself, either as secretions or excretions during cell life or inherent elements of the cytoplasm liberated after death.[53] They are produced in any medium in which the organisms grow, but to the greatest degree in the medium in which they grow best. Zinsser[53] believed that 'toxins are elements of intracellular metabolism, permanently or transiently constituent parts of the cell body.' Some toxins aid the bacteria to a considerable degree in maintaining themselves by destroying or injuring the tissues in which the bacteria grow. In such instances, the toxins may be considered as part of the offensive mechanism of bacteria against the host. But frequently, the action of the toxin takes place at a considerable distance from their focus of growth activity, and by destroying the host they effect eventually their own destruction. This will be discussed further below. Some toxic substances attack all of the cells uniformly; others possess a selective affinity for certain classes of cells. The latter act in very small doses because they are not dissipated.[54] Bacterial poisons may do their damage at the place of excretion from the body, as, for example, the poisons of typhoid and dysentery.[55] The selective

affinities of certain cells for certain poisons, as for instance the central nervous system for tetanus toxin, depend upon the relatively high coefficient of absorption possessed by these cells for the toxic substances. If none of the cells of the body possesses a particular coefficient of absorption, the substance is distributed uniformly throughout all the cells. If the coefficient of absorption is negative, no fixation of the poison occurs and it may be eliminated without change.[36] Certain substances may be toxic for the cells of one animal and not for another, just as they are toxic for certain organ cells within many species and not for other organ cells. D'Herelle believes that the primary criterion of pathogenicity depends upon whether or not the products resulting from the activities of the bacteria exercise a toxic action upon the leukocytes of the animal If this action does not take place, the organisms are quickly removed by the leukocytes.[37]

A. TRUE TOXINS

The so-called true toxins are poisonous substances obtainable by filtration of the culture medium in which certain bacteria have grown, before any large number of the bacteria have died or disintegrated. True toxins have been demonstrated for only a few species, *Clostridium tetani, C. botulinum, Pseudomonas pyocyanea, C. welchii, C. novyi, C. septicum, C. sordellii, C. chauvei, Corynebacterium diphtheriae,* and to a lesser degree, for the cholera, Shiga dysentery, and typhoid organisms; and also the hemolytic streptococci of scarlet fever and erysipelas. The hemolytic action of certain organisms is also due to a true toxin. Because these toxins are given off into the surrounding medium, they have been called exotoxins.[38] They possess certain common biologic characteristics, which they share with a number of nonbacterial substances, namely, snake venom, spider poison, and certain vegetable poisons like ricin. The peculiar characteristics of these poisons are that they are all soluble and more or less thermolabile and when injected into animals produce neutralizing antibodies called antitoxins. In these respects, they are sharply differentiated from the toxins liberated by bacteria when they die or the toxins extracted from bacteria. The latter are considered to be poisons within the body cell and are, therefore, called endotoxins.

Exotoxins are analogous to enzymes, but they are not catalytic, for they become permanently fixed to the substances on which they act. Most true toxins have a latent period of action when they are injected into animals.[39] In susceptible animals, toxins disappear from the circulation after injection, being fixed by the tissues to which they have an affinity. In nonsusceptible animals, they may remain in the circulation for some time, as, for instance, tetanus toxin in lizards.[40]

B. OTHER TOXIC PRODUCTS OF BACTERIA

1. *Endotoxins*

There seems to be little doubt that the great host of pathogenic bacteria that do not produce true soluble toxins in young cultures still produce substances having a poisonous action on the cells of the body. Pfeiffer[41] conceived these as being held, as such, within the bacterial body, and being released upon the death of the bacteria. Therefore, he designated them endotoxins and considered them specific for each organism. They are developed in older cultures when certain of the bacteria have died and disintegrated. When injected into animals, they have a profound toxic effect, but do not produce antitoxin. Vaughan[42] later expressed his belief that they are not specific poisons present as such in the bacterial cell and liberated after death, but that they result from split products of the bacterial protein. He believed that many of these are not specific and that similarity of clinical symptoms in diseases produced by different organisms favors this theory. The formation of these poisons is still not understood.

2. *Ptomaines*

Products of protein or lipoid cleavage produced by the action of putrefactive organisms on dead organic matter are called ptomaines.[43] This action usually takes place outside the body, and usually ptomaines poison the body when they are ingested with food. The ptomaine may, however, form to some extent by the putrefactive action of certain intestinal bacteria on food in the alimentary canal, or in necrotic tissue such as occurs in gangrenous extremities or in foul infections of the peritoneal cavity and abdominal wall following perforative lesions of the gut.

3. *'X' Substances*

Zinsser, Parker, and Kuttner[44] observed certain soluble, filtrable poisons in young cultures of a number of organisms that produce a slow death in laboratory animals when repeatedly injected. These are not specific poisons and apparently are similar in all of the bacterial species from which they were obtained.

4. *Poisons Produced by the Action of Bacteria upon Tissues within the Body*

As yet, these have not been demonstrated, because of the extreme difficulty of detecting or measuring such poisons either in the body or in tissue cultures; but that such poisons are formed when tissue is killed and is acted upon by the enzymes of the bacteria cannot be denied. These

poisons may, of course, not be specific for the organism. D'Herelle[45] observes: 'It is by no means astonishing that certain bacteria which secrete substances in the body possessing a violent toxic action, do not secrete them in an artificial medium.'

5. *The Offensive Mechanism of Bacteria*

It has been customary in discussions of infectious diseases to speak of the actions and reactions of the organism and the body as a 'battle' or a 'struggle,' and to conceive of each side marshaling its forces of offense and defense as a general does his army, as if there were indeed some motive in each phase of the process. It is obvious that, after all, the reactions are simply chemical and physical alterations due to fundamental laws that necessitate changes when two living organisms come into contact and carry on their life processes in the same place or in juxtaposition. If some of these reactions did not result in the survival of each of these at different times, one of the species would have been exterminated long ago. The only purposeful actions that the host brings into play are certain forces outside of his body cells—summed up in the word 'treatment.' Nevertheless, the conception of a struggle for existence is widespread, and if we acknowledge the limitations of the analogy, we may for purposes of clarity discuss the reactions of bacteria as if they were indeed offensive and defensive.

A. MOTILITY

Certain bacteria have the property of motility, owing to the action of small hairlike structures called flagella The motility does not necessarily correspond with virulence, and it is questionable whether the flagella actually play a role in the transportation of bacteria from place to place. If they do, however, motility may be considered as a mechanism of offense. It may also render the organisms more susceptible to the influence of forces like surface tension and electrical conductivity. On the other hand, the flagella may interfere with the passage of the organisms through narrow tissue spaces or vessel walls.

B. SPREADING FACTOR

In 1928, Duran-Reynals demonstrated in extracts from normal testicles a factor that increased the extent of the spread of a dye when injected into the skin of an animal[46] (Fig. 2) He later showed that extracts of certain bacteria had the same effect, and their presence could be correlated with the invasiveness of the organism. In a study of over fifty strains of staphylococci, he was able to divide the staphylococci into three groups according to the amount of this substance extractable from the washed cultures. This substance nonspecifically increased the inva-



FIG 2. The spreading factor of Duran-Reynals made manifest in the skin of a rabbit. A. The large lesion was produced by the intracutaneous injection of 0 5 cc. of *autolysate from an invasive strain of staphylococcus plus 0.25 cc. of India ink dilution.* B. Lesion produced by 0 5 cc. of the supernatant fluid of a centrified culture plus 0.25 cc. of India ink C. The small lesion produced by 0 5 cc. of water plus 0.25 cc. of India ink. (Courtesy of Dr F. Duran-Reynals)

siveness of nonpathogenic organisms. He believed that it acted by increasing tissue permeability.[47]

Later, Hobby, Dawson, Meyer, and Chaffee[48] tried to identify the ferment hyaluronidase with this spreading factor. Hyaluronidase lowers the viscosity of hyaluronic acid, which gives the mucinous character to various tissues and body fluids, including synovial fluid and vitreous humor. They concluded that spreading is not a single phenomenon but a group of reactions depending on a variety of mechanisms one of which may be hyaluronidase activity.

McClean and Rogers[49] have shown that hyaluronidase-producing aerobes, mixed with nonhyaluronidase anaerobes, will cause the latter to spread more rapidly and invade more extensively. They have suggested that the demonstration of these specific enzymes in wound exudates will permit an early diagnosis of the activity of certain organisms like streptococcus, staphylococcus, or the clostridia of gas gangrene.[50]

C. TOXICITY

Other organisms tend to remain local but effect their deleterious action on the body by the production of poisons that are carried through the

blood stream to the susceptible tissue or organ and actually unite with the tissue. That these toxins are absorbed by the susceptible tissue has been repeatedly demonstrated. Red cells absorb hemolysin from a culture, and cells of the central nervous system will absorb tetanus toxin. The tetanus bacillus, the diphtheria bacillus, and the streptococci of erysipelas and scarlet fever are examples of organisms whose effect is primarily toxic. The bacteria of the latter group are rarely found in the blood, but frequently produce death.

D. ANTILEUKOCYTIC ACTION

Organisms are frequently found within the motile or fixed cells of the body, and we say that they are 'phagocyted.' It is definitely known, however, that in certain cases the bacteria are not destroyed within the phagocytes but multiply and in their turn destroy them. In such cases, we cannot be sure that the bacteria do not in fact invade the cells. Bacteria may actively resist phagocytosis by producing a substance that repels the phagocytes, by neutralizing their digestive ferments, or actually digesting them. The growth of bacteria within phagocytes is illustrated by Figure 3. The repulsion of leukocytes is seen in Figure 1.

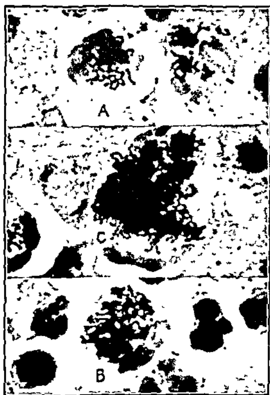


FIG 3 Showing several stages in a case of a mixed streptococcus and staphylococcus infection reported by Drs Brewer and Meleney. A. The leukocytes contain a few chains; B. The leukocyte is stretched by the mass of chains, C. The organisms have continued to grow and there are nuclear fragments about the tangled mass. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

E. CYTOLYTIC ACTION

Unquestionably, tissue cells die in the presence of bacterial infection, but it has not yet been definitely determined whether this is a direct action of the bacteria on the cell protoplasm or a secondary action resulting from a cutting off of the nourishment of the cell by effecting some alteration of the surface or by blocking a vessel at some distance from the cell. The action of certain organisms, as for example, *Clostridium histolyticum*, as shown on page 256, can hardly be other than a complete digestion of the tissue cells. Alsberg[51] points out that substances may affect cells in three different ways: (1) by attacking the surfaces, either by precipitating, coagulating, or dissolving constituents of the surface or by coming into chemical or physical union with these surface layers, (2) by entering the cell and causing chemical or physical alterations within the protoplasm; (3) by altering the concentration within the cells. Thus the reaction of an extraneous substance with a cell must depend not only upon its chemical but also upon its physical properties.

F. 'AGGRESSINS'

Bail[52] originated the term 'aggressins' to designate certain substances secreted by virulent bacteria but not by avirulent strains. They are produced only under the stress of a struggle against body defense. Bail could not demonstrate them in test-tube cultures. They may be obtained from virulent bacteria by injecting these strains into the peritoneum of a guinea pig and after the guinea pig dies, removing and centrifuging the exudate. The supernatant fluid is then treated with chloroform to kill stray organisms. The fluid causes avirulent strains to become virulent, and virulent strains to become more rapidly lethal. Levy and Fornet[53] later showed that a number of bacterial filtrates have aggressive substances that are nonspecific in helping nonvirulent organisms to become virulent. These substances have the same function and may be identical with the substances now called spreading factors, including hyaluronidase, mentioned above.

6. *The Defensive Mechanism of Bacteria*

A. PHYSICAL RESISTANCE

Bacteria vary considerably in their resistance to physical changes in their environment. Some are readily destroyed by exposure to sunlight or to drying or to even moderate degrees of heat above their maximum-growth temperature. Most are more resistant to cold and may be preserved for a long time in temperatures just above the freezing point.

However, alternate freezing and thawing may cause a physical disruption of the organism.

B. CAPSULE FORMATION

Many organisms develop capsules, and it is seen in the body that these resist phagocytosis; or, if they are ingested by the organism, they resist the intracellular digestion to which noncapsulated forms succumb. It has been noted by Lyons[54] and others that some organisms form capsules under certain conditions and fail to form them under others. For example, some virulent staphylococci are encapsulated in the early stages of their cultivation and noncapsulated later. While they are in the encapsulated state, they have antigenic properties that they later lose. In general, encapsulated organisms are more virulent than noncapsulated ones and they are more resistant to the phagocytosis of leukocytes. The tubercle bacillus is believed to have a waxy capsule, which increases its resistance to the action of the body cells.[55]

C. SPORULATION

Many organisms form spores and become particularly resistant to heat and dryness. Certain anaerobic strains, particularly of *Clostridium sporogenes*, have been found to resist boiling for an hour[56] Most spores survive a temperature of 80° C. for fifteen minutes, which temperature destroys most vegetative forms The spore form of some organisms may live for months or years in a dried state and still longer in the cold.

D NEGATIVE CHEMOTAXIS

Certain organisms have been demonstrated to produce substances that repel leukocytes, reversing the usual force of chemotaxis.[57] D'Herelle[58] states that 'all bacteria which exert a negative chemotaxis toward the leukocytes of any given animal are pathogenic for this animal. . . But the reverse is not true'—that is, many bacteria that attract leukocytes are still pathogenic. It is impossible to state whether this leukocyte-repellent action is defensive or offensive. Tetanus spores that are washed attract leukocytes and are phagocyted unless there is some injury to the tissue at the point of inoculation. Tetanus spores plus tetanus toxin repel leukocytes, and disease ensues.

It has been demonstrated that bacteria increase in virulence for a given species by repeated inoculation. This increase in virulence has been accompanied not only by evidence of the production of aggressive substances but also by the development of certain protective substances, and Welch[17] suggests that within the bacterial cell there may be a development of an immunity similar in character to the general immunity developed by the host against an invading organism.

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CHAPTER XIII

Bacterial Spread through the Human Body

I. SPREAD BY SIMPLE INCREASE IN NUMBERS

IN ORDER that bacteria may first gain a foothold, as has been shown in the preceding chapter, they must be able to resist the defense of the body long enough to grow and multiply. They multiply to a variable degree, depending upon the extent to which their offensive and defensive forces outweigh the offensive and defensive forces of the body. It has been found that in the most favorable culture media bacteria multiply at the rate of about one generation every twenty to thirty minutes. The rate of growth in culture media is not uniform, but follows a curve. There is at first a period of lag, in which the numbers fall off to some extent, and then a rapid increase for a period of 8 or 10 hours. This is followed by a stationary period, and later a fall in the number of viable organisms.[1, 2] In artificial media, it has been demonstrated that the products of bacterial growth inhibit development in the later stages. A change in the reaction of the medium toward the acid side will delay the growth of most organisms.

In the body, the rate of growth is hard to determine because of the difficulty of estimating the inhibiting power of the host and the speed with which the products of bacterial metabolism are carried away from the site of growth. It is obvious that bacteria do increase enormously in numbers, for in almost every case of infection the initial introduction of bacteria is small, and when the infection has developed to a considerable extent, bacteria are found widely distributed, even locally, from the point of entrance. But in infected foci, there is no such prolific multiplication of organisms as takes place in artificial media or in the intestinal tract, and the actual bulk of organisms, even in extensive infections, represents but a small fraction of the increase in the total mass of the inflamed part. In acute processes with abscess formation, the greatest concentration of organisms is in the pus, although many of these are doubtless dead. From the walls of the abscess outward the concentration decreases steadily, until at the margin, where there are edema and cellular infiltration, no organisms may be found either by culture or tissue

staining. On the other hand, in rapidly spreading erysipelas, it has been found that as the margin spreads and the center cools, viable organisms may be found beyond the red margin, while cultures from the older central portion are negative.[8]

II. DIRECT SPREAD

The degree to which the mass of bacteria increases depends, to some extent, upon the character of the tissue in which they are growing. Very dense tissues, such as bone, or even the thick tissues of the back of the neck or breast, do not permit the spread of the organisms to any degree. The pressure exerted by the increasing mass of bacteria naturally takes the direction of least physical resistance, so that the organisms will be pushed along through the softer tissues, such as the medullary cavity in bone, or along the ducts and the acini between the main connective-tissue septa in the breast. In the skin of the neck, their tendency is first to go deep and then laterally along fascial planes. In like manner, the actual growth of the original focus may cause a spread from one tissue to a contiguous tissue, or from one organ to another organ, or from an organ to a neighboring viscus. In general, a spread of this kind is from the more dense to the less dense tissue. Direct spread can best be illustrated by autopsy material in cases seen before the advent of sulfonamides and penicillin. See the following Cases I, II, and III.

Case I: E.M. Age 34. History no 44959. Autopsy no. 8992.

HISTORY. Twenty-four hours before admission, the patient was suddenly seized with chills, marked prostration, and fever. Headache and general bone and joint pain developed and rapidly increased in severity. Her throat became sore, and a cough developed. Loss of appetite and nausea and vomiting ensued.

PHYSICAL EXAMINATION. The patient was a well-nourished, middle-aged woman, acutely ill. Her face was flushed and her skin warm and dry. The pharynx was red, but the tonsils were not inflamed. The cervical glands were slightly enlarged. The heart was normal. The lungs exhibited harsh breath sounds and scattered, sonorous râles, but there seemed to be no involvement of the alveoli. The abdomen and extremities were negative. The temperature on admission was 100.8° F., the pulse rate 120, and the respirations 20. A diagnosis was made of 'influenza' with bronchitis.

COURSE. On the fifth day, the patient's temperature began to mount, and on the sixth day, signs of consolidation appeared for the first time in her left-lower lobe, with pleuritic pain. A blood culture on that day was negative. On the seventh day, signs of fluid appeared at the left base. Aspiration produced moderately thick, purulent fluid containing many Gram-positive cocci in chains. The culture revealed hemolytic streptococcus. A white blood count on this day showed 18,000 white cells, with 95 per cent polymorphonuclears A thoracotomy was done and closed drainage instituted. The lung was not

adherent to the costal pleura. After operation, the patient became cyanotic and dyspneic. The pulse became very rapid, 148, and respirations 36. The temperature rose to 103.2° F., and the patient expired.

AUTOPSY. The lateral surface of the left lung was covered by a thick, fibrinous exudate. The apex of the lung was adherent to the wall by fresh adhesions. Elsewhere, it was free and separated from the parietal pleura by purulent fluid. The right upper lobe was adherent by old adhesions, but there was no fluid on the right side. The left lung showed a diffuse consolidation of the lower lobe, with small hemorrhages and small abscesses just beneath the pleura. The upper lobe was aerated. The right lower lobe showed patchy areas of consolidation. The pericardium showed numerous small hemorrhages. The heart was relatively normal The peritoneum and its contents were relatively normal. Microscopically, the left lung showed consolidation of the alveoli with purulent exudate and multiple miliary abscesses, many of which were subpleural. These contained numerous chains of Gram-positive cocci.

This case illustrates the entrance of bacteria into the respiratory tract, probably along the bronchi, reaching the periphery of the lungs, forming abscesses there, and then by direct extension passing into the pleural cavity (Fig 1).

Case II· G C. Age 62. History no. 56875. Autopsy no. 9389.

HISTORY. Five days before admission, the patient developed a sore throat, which rapidly became worse. Then followed chills and high fever. His breathing became difficult the day before admission and was increasingly labored up to the time of admission.

PHYSICAL EXAMINATION. The patient was in great distress, breathing with difficulty, both during inspiration and expiration The veins of the neck were engorged. The throat was acutely inflamed. The chest and abdomen were negative The temperature on admission was 103 2° F., pulse rate 126, and respirations 32

COURSE An immediate tracheotomy was done under local anesthesia, with considerable relief from the dyspnea A blood culture revealed hemolytic streptococcus. Following operation, the temperature rapidly rose to 105° F., and the patient relapsed into a semicomatose state, from which he did not recover. On the third day, his temperature reached 106 2° F., his pulse rate 130, and his respirations 50. Signs of consolidation appeared in the right lung, and death ensued.

AUTOPSY. An abscess was found around the larynx, with an ulceration of the mucous membrane on the left side about 1 cm in diameter. Two smaller ulcerations were present on the right side directly opposite. There were cellulitis of the neighboring tissues and acute inflammation of the thyroid gland The pericardium contained 50 cc. of slightly turbid, yellow fluid. The lungs showed a fibrinous exudate over both lower lobes, and on section the lower lobes presented patchy areas of consolidation The bronchi contained frothy, yellow fluid. The peritoneum and abdominal organs were relatively normal. Microscopic examination showed the wall of the laryngeal abscess to be densely



FIG 1. CASE I: Hemolytic streptococcus empyema. Subpleural abscess of lung, × 200, showing some exudate on surface. *Insert* Streptococci in abscess of lung, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)



FIG. 2. CASE II: Hemolytic streptococcus Ludwig's angina. Abscess of larynx, showing surface epithelium and abscess wall, × 95 *Insert:* Streptococci in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 3 CASE II: Acute thyroiditis. (Photo by Dr. W. C. von Glahn) *Insert* Streptococci in thyroid, × 1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

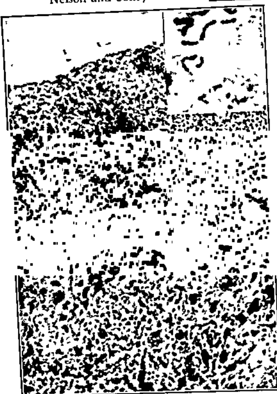


FIG 4 CASE II. Consolidation of lung and pleural exudate, × 150 *Insert* Streptococci in pleural exudate, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

infiltrated with polymorphonuclear leukocytes with a large, central area of necrosis. There were numerous Gram-positive cocci in chains. The mucous membrane of the trachea and bronchi showed acute inflammation, with some surface necrosis and cellular exudate. The thyroid showed many areas of early abscess formation with Gram-positive cocci. The consolidated areas in the lungs and the exudate on the pleura were loaded with polymorphonuclear cells and Gram-positive cocci.

This case illustrates the entrance of bacteria into the physiologic interior of the body at the upper part of the respiratory tract and their direct spread to the tissues and organs of the neck. The spread was also downward to the deeper portions of the respiratory tract, either directly through the bronchi or along the bronchi in the deeper tissues, and thence outward through the pleura (Figs. 2-4).

Case III. H.M. Age 58. History no. 57762. Autopsy no. 9419.

HISTORY. Ten months before admission, the patient began to have very severe knifelike pains in the right upper quadrant of the abdomen. The pain was localized, and the area was extremely tender. Jaundice appeared a few days after the first attack and lasted for six weeks, varying in intensity. The attacks would last for fifteen minutes to an hour They gradually became less intense and finally disappeared. After several months, attacks of pain and jaundice recurred at frequent intervals. The last attack, which was six days before admission, was preceded by a chill.

PHYSICAL EXAMINATION. The patient looked acutely ill. He was markedly jaundiced. The heart and lungs were normal. The abdomen showed marked spasm of the upper right rectus and the oblique muscles, with marked tenderness on pressure. A large mass was present in the right upper quadrant. The lower margin of this mass could not be clearly defined. On admission, the temperature was 103.6° F., the pulse rate 98, and the respirations 24. The white blood cells were 19,400, with polymorphonuclears 90 per cent. The blood-clotting time was prolonged. The blood urea was normal.

COURSE. For three days the patient ran a high, even temperature. On the fourth day, he was operated upon, at which time an extensive, inflammatory process in the right upper quadrant made a definition of the anatomic landmarks quite difficult. The omentum was drawn up over the duodenum and gall bladder and when it was separated, an abscess was found between the duodenum and liver containing foul-smelling pus and a number of gallstones The gall bladder could not be distinguished in this mass of tissue. A round stone was found in the common duct, which also contained many smaller calculi, pus, and bile. The common duct was drained. The culture of the pus showed *Bacillus coli* The patient progressed favorably for three days. On the third day he was given charcoal by mouth and it appeared in the drainage tract, indicating that there was a duodenal fistula. That evening he developed signs of peritonitis and a jejunostomy was performed, but he failed rapidly and died on the third day after the second operation.



Fig. 5. Case III: Direct extension of bacteria from duodenum into gall bladder and thence into liver following gallstone fistula between gall bladder and duodenum. Abscess of liver contiguous to gall bladder. (Photograph by Dr. W. C. von Glahn. From Nelson's *Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)

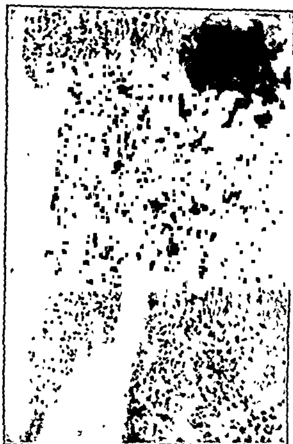


FIG. 6 CASE III: Wall of gall bladder, × *100*. *Insert:* Mixture of organisms in gall bladder, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

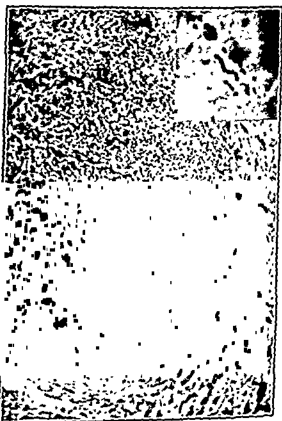


FIG. 7. CASE III: Abscess of liver, × 50 *Insert:* Streptococci in liver abscess, × 1500 (Photograph by Dr. W. C. von Glahn From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

AUTOPSY. The body was greatly emaciated. The skin and sclerae were yellow. When the abdominal cavity was opened a general peritonitis was found, with the center of the process apparently in the right upper quadrant. In the liver were multiple small abscesses, particularly around the portal vessels. The gall bladder was thickened and contracted, and at the apex of the gall bladder was a small opening with fairly smooth edges leading into a large abscess cavity in the liver. There was also a fistula between the gall bladder and duodenum. The common duct contained two stones. When the thoracic cavity was opened, the left lung was found to be adherent to the pericardium and to the diaphragm. On section both lungs showed a number of small abscesses. Microscopic examination showed necrotic tissue in the walls of the abscesses in the liver and in the lungs. In the gall bladder, both cocci and bacilli were found, whereas in the liver-abscess wall were found large numbers of streptococci, but no bacilli.

This case illustrates the possibility of direct extension of bacteria from duodenum into gall bladder and thence into the liver in a case of gallstone duodenal fistula (Figs. 5-7).

III. INTRAVENOUS SPREAD

Bacteria may also spread along the walls of blood vessels with the propagation of a thrombus, by simple direct extension, either in the direction of the blood stream or retrograde, as in Case IV.

Case IV: D.C Age 18 History no. 47728. Autopsy no. 9068.

HISTORY. Sixteen days before admission, the patient was operated on for a left peritonsillar abscess after a four-day history of fever and sore throat. Following this, he improved for two days and then his neck began to swell. Six days later, the left peritonsillar abscess was incised again, with local relief A few days after the second operation, his right eye began to swell and he suffered terrific headaches with deafness and buzzing in the ears. Fever and sore throat returned, and a cough developed, with blood-tinged sputum. Four days before admission, he became delirious and was brought to the hospital in that state.

PHYSICAL EXAMINATION. On admission, the patient was found to be prostrated, with flushed face and slight icteric tinge to the skin. The right eye was swollen and protuberant. The upper eyelid was red and swollen, and the conjunctiva edematous. The left tonsil, uvula, and left pharyngeal wall were covered by a membrane. The left side of the neck was greatly swollen, tender, and very hot, but not red. There was marked rigidity of the neck, partly as a result of the swelling A friction sound was heard near the apex of the heart. The lungs showed small, patchy areas of consolidation The abdomen was tender all over. The spleen was palpable. On admission, the temperature was 105.2° F., the pulse 100, the respirations 24. The white blood count was 15,500 with polymorphonuclears 86 per cent. A blood culture was negative. Examination of the fundus of the right eye showed marked dilatation of the veins.

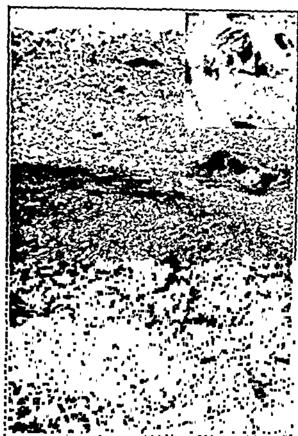


FIG. 8. CASE IV: *Proteus vulgaris* peritonsillar abscess followed by septicemia Thrombosis of jugular vein, × 15 *Insert* Gram-negative bacilli in thrombus, × 1500. (*From Nelson's Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)



FIG. 9. CASE IV: Thrombosis of lateral sinus, × 15. (*From Nelson's Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)

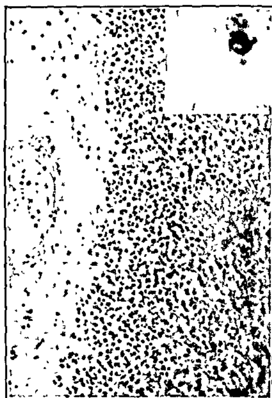


FIG 10 CASE IV: Acute bacterial encephalitis, × 150 *Insert:* Gram-negative bacilli in exudate on surface of brain, × 1500 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

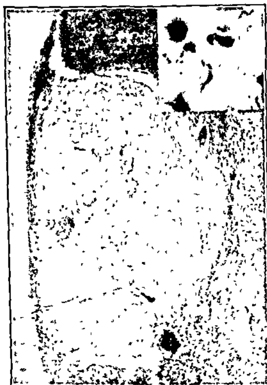


FIG 11 CASE IV: Abscess of lung, × 10 *Insert* Gram-negative bacilli in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery*. *Courtesy of Thomas Nelson and Sons*)

COURSE. The patient very rapidly passed into coma. His temperature rose to 108° F., and he died.

AUTOPSY. The right half of the face and neck were very edematous. The right eye was slightly protuberant, and the sclerae were slightly yellow. The uvula was very edematous. The left tonsil was not greatly enlarged, but contained numerous deep crypts. Below the left sternomastoid muscle and extending up anteriorly to the ear and then over the angle of the jaw was a large abscess cavity that contained chocolate-colored fluid. The jugular vein was surrounded by a large mass of edematous lymph glands, which formed one wall of this abscess cavity. The jugular vein contained a soft, infected thrombus, which extended upward into the lateral sinus, the left inferior petrosal sinus, and also the left sigmoid sinus. Both the cavernous and the circular sinus and the left petrosal sinus, as well as both ophthalmic veins, were filled with greenish-yellow pus, and the posterior orbital tissues, particularly of the right eye, were edematous. On the base of the brain, there was a thick, purulent exudate The pleural cavities were relatively normal. Both lungs showed marked congestion of the posterior and inferior portions and small areas of consolidation. In the left lung, there was a small subpleural abscess. The pericardium was normal except for a few petechial areas on the surface of the heart. The heart was otherwise relatively normal. A blood culture at autopsy yielded *Proteus vulgaris*. This finding was considered to be a contamination until the microscopic examination was made of the tissues. The abdominal contents appeared normal. Microscopically, the thrombus in the jugular vein in the neighborhood of the abscess of the neck showed an acute inflammatory lesion, with great numbers of polymorphonuclear leukocytes. Gram stains of the purulent exudate in the jugular vein and in the sinuses of the dura showed short, Gram-negative bacilli in great numbers. Some were also found in the exudate on the brain, in the wall of the peritonsillar abscess, and in the abscess of the lung.

This case illustrates the entrance of bacteria through the tonsils into the tissues of the neck, with a thrombosis of the jugular vein and an invasion by bacteria of the clot. Then a retrograde spread occurred upward along the vein into the sinuses of the dura, and thence outward to the meninges of the brain. The case illustrates also a spread centrally to the lung, probably by way of the vein, but possibly by the bronchi. *Proteus vulgaris* is rarely pathogenic, but the evidence seems to be that it was the causative organism in this case (Figs. 8-11).

IV. SPREAD BY BACTERIAL MOTILITY

A. MOTILITY OF BACTERIA

This has been discussed briefly in Chapter XII, page 393.

V. SPREAD BY EXTRAVASCULAR CIRCULATING FLUIDS

A. INFLUENCE OF THE CIRCULATION OF FLUIDS ON THE SPREAD OF BACTERIA

In the normal body, fluid is in constant motion in the tissue spaces, as well as in the lymph and blood channels. Bacteria in the tissue spaces may be mechanically carried along by the circulating fluid. The lymph and *intracellular tissue juices make their way back into the lymphatics* and to a certain extent into the capillaries also, although the pressure within the capillaries largely directs the flow of fluids outward. There has been considerable debate with regard to the nature of the lymphatic terminals. If they were open to the intercellular spaces, the bacteria would have easy access to them. The weight of opinion, however, favors the belief that the distal ends of lymphatic vessels are closed,[4] and that, in order to enter them, bacteria must pass through an endothelial wall. This doubtless offers at least a temporary barrier to the spread of bacteria by way of normal lymphatic or venous channels. But whenever lymphatics or capillaries become blocked or involved in an inflammatory process, the walls are injured, thrombi form, organisms or leukocytes liquefy tissues, including vessel walls, and the organisms find themselves on the interior of the circulatory system and under the influences of the current. Thus they may be carried up the lymphatics or veins.

VI. SPREAD BY INTRAVASCULAR CIRCULATING FLUIDS

Bacteria entering lymphatics may be caught in the regional lymph glands and there either be destroyed or set up a metastatic focus of infection. To some extent, doubtless, the thromboses occurring in the vessels of an infected focus at first tend to hold the bacteria locally, but they also interfere with the defensive mechanism of the body and result in further death of tissue. When a vessel becomes thrombosed, the thrombus not infrequently becomes infected. The action of the bacteria upon the clot tends in most cases to soften it and render it pliable and separate it from the vessel wall. Then any movement of the part may cause it to break off. If it is in a vein, a large mass of infected blood clot may be carried to the heart and lungs. The infected embolus will be stopped at a point where the blood channels become too small for it to pass. Probably most of these rest in the lungs, but some small septic emboli, and certainly individual bacteria, may pass directly through the circulation of the lungs, returning again to the heart, and be carried through the arterial circulation until they are stopped by a vessel too small to permit their passage. Wherever these emboli stay, the organisms that are present are subjected to the new environment. This environment

may or may not be a favorable place for their continued growth and multiplication. If it is not, they perish; if it is, this region becomes a new focus of infection, from which the organisms may spread in the same manner in which they spread from the original point. If this secondary focus be a heart valve, the vegetations growing thereupon are subject to the intermittent forceful movements of the blood current, which doubtless cause an intermittent discharge of bacteria into the blood stream. Usually, extensive metastases then develop, and death ensues. This is illustrated by Case v.

Case V: J.P. Age 25. History no. 58981. Autopsy no. 9469.

HISTORY. Two weeks before admission, the patient's appetite became poor. Four days before entrance, he had general malaise and a sense of weakness. The next day, chills, fever, and headache appeared; and two days later, after an interval of relative improvement, he began again to have fever and a cough, with blood-tinged sputum. Hemorrhagic spots appeared on the skin. On the day of admission, a paralysis of the left arm developed. The patient was too sick for a complete history to be obtained from him.

PHYSICAL EXAMINATION. The patient was an extremely ill but well-developed and well-nourished young man. His respirations were rapid and irregular. He coughed from time to time and brought up blood-tinged sputum. There were many petechial hemorrhages of various sizes in the skin and conjunctiva. The lungs revealed a few râles and bronchovesicular breathing in the right anterior chest and at the right base, with dullness. The heart was enlarged to the left. The action was regular but rapid, with feeble heart sounds. The abdomen was soft The liver was large, and a friction rub could be felt. The spleen was not palpable. There was partial paralysis of the left arm and some spasticity of the right arm and both legs. The temperature was 105.2° F., pulse rate 110, and respirations 40 A white blood count showed 11,000, with polymorphonuclears 91 per cent. The blood pressure was 75/45. A blood culture yielded hemolytic *Staphylococcus aureus*.

COURSE. On the second day, the blood count rose to 53,000 white cells, with 91 per cent of polymorphonuclears. The temperature mounted to 105.4° F., and the patient died.

AUTOPSY Petechial hemorrhages were found scattered over the body. They were particularly numerous around the eyelids, on the conjunctivae, and on the hands. There were also large areas of hemorrhage in and beneath the skin of the back and chest, one of the areas being 10 cm. in length. The thoracic cavity showed normal pleurae The pericardial sac contained 25 cc. of turbid fluid, in which were a few flakes of fibrin. The heart showed numerous petechiae on the surface and extensive vegetations on the mitral valve, which almost occluded the orifice and were attached to both surfaces of the valve leaflets Smaller vegetations were present on the aortic valve. Scattered throughout the lungs were small consolidated areas with central necrosis, particularly in the lower lobe. The spleen showed a number of infarcts, with yellow areas of necrosis in the center. On the anterior surface of the liver were



FIG. 12. CASE V. Cryptogenic *Staphylococcus aureus* septicemia, probably starting in the prostate gland. Abscess of prostate, × 150, showing a relatively old process. *Insert* Staphylococci in prostate abscess, × 1500. (*From* Nelson's *Loose-Leaf Surgery*. Courtesy of *Thomas Nelson and Sons*)



FIG 13. CASE V. Thrombosis of iliac vein, × 200. *Insert* Staphylococci in thrombus, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

may or may not be a favorable place for their continued growth and multiplication. If it is not, they perish; if it is, this region becomes a new focus of infection, from which the organisms may spread in the same manner in which they spread from the original point. If this secondary focus be a heart valve, the vegetations growing thereupon are subject to the intermittent forceful movements of the blood current, which doubtless cause an intermittent discharge of bacteria into the blood stream. Usually, extensive metastases then develop, and death ensues. This is illustrated by Case v.

Case V: J.P. Age 25. History no. 58981. Autopsy no. 9469.

HISTORY. Two weeks before admission, the patient's appetite became poor. Four days before entrance, he had general malaise and a sense of weakness. The next day, chills, fever, and headache appeared; and two days later, after an interval of relative improvement, he began again to have fever and a cough, with blood-tinged sputum. Hemorrhagic spots appeared on the skin. On the day of admission, a paralysis of the left arm developed. The patient was too sick for a complete history to be obtained from him.

PHYSICAL EXAMINATION. The patient was an extremely ill but well-developed and well-nourished young man. His respirations were rapid and irregular. He coughed from time to time and brought up blood-tinged sputum. There were many petechial hemorrhages of various sizes in the skin and conjunctiva. The lungs revealed a few râles and bronchovesicular breathing in the right anterior chest and at the right base, with dullness. The heart was enlarged to the left. The action was regular but rapid, with feeble heart sounds. The abdomen was soft The liver was large, and a friction rub could be felt. The spleen was not palpable. There was partial paralysis of the left arm and some spasticity of the right arm and both legs The temperature was 105.2° F., pulse rate 110, and respirations 40. A white blood count showed 11,000, with polymorphonuclears 91 per cent. The blood pressure was 75/45. A blood culture yielded hemolytic *Staphylococcus aureus*

COURSE On the second day, the blood count rose to 53,000 white cells, with 91 per cent of polymorphonuclears. The temperature mounted to 105.4° F., and the patient died.

AUTOPSY. Petechial hemorrhages were found scattered over the body. They were particularly numerous around the eyelids, on the conjunctivae, and on the hands. There were also large areas of hemorrhage in and beneath the skin of the back and chest, one of the areas being 10 cm. in length. The thoracic cavity showed normal pleurae The pericardial sac contained 25 cc. of turbid fluid, in which were a few flakes of fibrin The heart showed numerous petechiae on the surface and extensive vegetations on the mitral valve, which almost occluded the orifice and were attached to both surfaces of the valve leaflets Smaller vegetations were present on the aortic valve. Scattered throughout the lungs were small consolidated areas with central necrosis, particularly in the lower lobe. The spleen showed a number of infarcts, with yellow areas of necrosis in the center. On the anterior surface of the liver were



Fig 12 Case V: Cryptogenic *Staphylococcus aureus* septicemia, probably starting in the prostate gland. Abscess of prostate, × 150, showing a relatively old process. *Insert* Staphylococci in prostate abscess, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig 13 Case V. Thrombosis of iliac vein, × 200 *Insert* Staphylococci in thrombus, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG 14 CASE V: Vegetations on the mitral valve (Photograph by Dr. W. C. von Glahn From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

many dilated vessels filled with a purulent fluid. On section, the liver revealed many small, yellow areas of suppuration The left kidney showed mottled, hemorrhagic areas on the surface beneath the capsule. Most of the hemorrhagic areas were yellow in the center. On section, these were found to extend down into the cortex for a variable distance. In the right kidney was a similar condition except that, in addition, the pelvis was found to be filled with turbid fluid, and a stone was present, filling two of the calices. The right lobe of the prostate contained an abscess from which thick, creamy, yellow pus flowed The veins on either side of the prostate were occluded by partially organized,

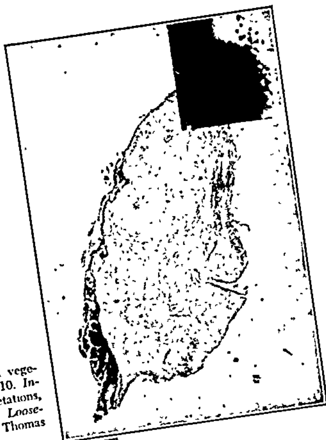


Fig 15. Case V: Bacterial vegetations on aortic valve, × 10. Insert: Staphylococci in vegetations, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



firm thrombi, which were not grossly purulent. The lymph glands along the iliac vessels were enlarged and contained small miliary abscesses, and in the receptaculum chyli and in the thoracic duct a bloody fluid was found, but there was no dilatation nor thombosis of the duct. The stomach and intestines showed a few hemorrhagic areas beneath the mucosa and the serosa.

Microscopically, the abscess of the prostate seemed to be an older process than the other abscesses found. Smears of the pus from the prostate showed Gram-positive cocci, and these were found microscopically in the tissue sections as well. Cultures of the pericardial fluid showed hemolytic *Staphylococcus aureus*. The thoracic duct failed to show any acute inflammation, but smears from the bloody fluid in the duct showed many Gram-positive cocci, and hemolytic *Staphylococcus aureus* was obtained by culture. The veins of the pelvis showed early organization of the thrombi. In one area, there was active inflammation, and Gram-positive cocci were present in the thrombus. Abscesses were found in the spleen, liver, and kidneys, all of which showed bacteria in great numbers. Small abscesses were also found in the heart and pancreas. The vegetations on the heart valves showed great masses of Gram-positive cocci. The lungs showed a few small areas of bronchial pneumonia, which contained diplococci.

The age of the process in the prostate and in the thrombi in the pelvic veins suggested that the primary focus of infection was in the prostate gland and the primary route of spread was venous The presence of cocci in the thoracic duct shows that the spread was also lymphatic, but without inflammation of the duct itself.

This case illustrates a cryptogenic hemolytic *Staphylococcus aureus* infection, with the primary focus probably in the prostate gland. The organisms spread thence to the neighboring veins, which became thrombosed. They spread also by way of the thoracic duct, without thrombosis. For some reason, there were no abscesses in the lungs, probably because no gross emboli came from the pelvic veins. It may be that the chief route of spread was via the thoracic duct, and the bacteria were either destroyed in the lungs or passed through to the arterial side. Vegetations appeared on the heart valves of the left side of the heart, and from those foci septic emboli spread to various organs of the body (Figs. 12-16).

VII. SPREAD FROM A CONTAMINATED SOURCE TO AN INJURED AREA

A very interesting group of cases comprises those in which thrombosis occurs in the pelvic veins without any suspicion of infection. Huge clots may break off and plug large branches of the pulmonary artery. Death is often dramatically sudden. In many cases, however, smaller particles of clot break off and produce multiple pulmonary infarcts. If the patient survives the first shock, gangrene or abscess of the lung not infrequently

ensues. This is more likely to occur if the clot is primarily infected at the site of its origin, but there seems to be definite evidence that sterile thrombi may first produce infarcts and that these become secondarily infected by organisms from above. The formation of multiple lung abscesses following presumably sterile pulmonary emboli is illustrated in Case VI.

Case VI: K.F. Age 73. History no. 44599. Autopsy no. 8983.

HISTORY. The patient had been sick for eight weeks, following a sudden attack of palpitation of the heart accompanied by swelling of the feet. She had had no previous attacks but after they began they had continued without improvement, even with rest and medication.

PHYSICAL EXAMINATION. The patient was acutely ill Her skin was cyanotic. She breathed with great difficulty. The veins of the neck stood out. The heart was considerably enlarged. Its rate was very slow (44), with occasionally coupled beats. There were soft systolic and diastolic murmurs at the apex. Both lungs were dull to percussion at the bases, with numerous râles, particularly on the left side. The abdomen was negative. There was marked edema of the left foot and leg, but the right side was only moderately edematous The temperature on admission was normal The white blood cells were 9600 with 79 per cent of polymorphonuclears. The urine showed albumin and casts. The blood urea was 0 83 gm per liter.

COURSE. An electrocardiogram revealed a complete heart block, which persisted The edema of the left leg did not clear up with rest in bed and elevation. The patient gradually became more dyspneic and cyanotic, and finally irrational. The breathing, too, was of a Cheyne-Stokes character During the last three days, the temperature gradually rose from normal to 101° F., and the patient died.

AUTOPSY. The body was greatly emaciated There were many petechial hemorrhages on the skin, and a papular eruption The left pleural cavity contained 100 mils of cloudy, blood-tinged fluid, with a number of old adhesions on the upper part of each lobe The right pleural cavity contained a smaller amount of fluid and fewer adhesions. The upper part of the left upper lobe was air-containing but the lower part and all of the lower lobe were solid. In the branches of the pulmonary artery leading to these areas, blood clot emboli were found. On section, the lung tissue was dark red in color with multiple abscesses, from which bloody, purulent fluid escaped. Part of the right lower lobe was similarly involved. One of the large branches of the pulmonary artery was filled with an embolus. The pericardium was normal The heart was enlarged Both ventricles were dilated. A thrombus was found in the left coronary artery. The mitral valve was small, and the cusps were stiff. The aortic valve was also thickened. A dissection of the pelvic veins revealed, on the left side, a complete obliteration of the femoral and iliac veins by an old thrombus. There seemed to be no evidence of infection of the clot. Microscopically, no organisms were found in the clot within the veins or in the large branch of the pulmonary artery. In the lungs, many infarcts were



FIG. 17. CASE VI: Lung abscess following pulmonary infarction. Thrombosis of femoral and iliac veins. An examination of the clot for bacteria revealed none. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

FIG 18 CASE VI: Thrombus in heart, × 15 (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)





Fig. 19. Case VI. Thrombus in pulmonary artery, × 15. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

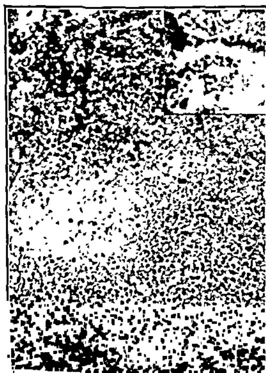


Fig. 20 Case VI: Abscess of lung, × 200 *Insert* Mixture of bacteria in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

found in all stages of formation. Some of these were organized but many of them were replaced by purulent foci and in the abscesses mixtures of organisms were found (Figs. 17-20).

This case illustrates the production of pulmonary infarcts by a supposedly sterile venous clot and subsequent infection and abscess formation in some of these injured lung areas probably due to organisms descending from the throat.

VIII. SPREAD BY LYMPHATICS

It is generally believed that bacteria more frequently enter lymphatics than veins. It is common enough for regional lymph glands to become *involved without any evidence of inflammation in intermediate lymph* channels. Subsequently, these glands may resolve or break down and form abscesses. At times, the surface lymphatic vessels become inflamed, and local abscesses may form along their course, in regions where lymph glands are not normally described by anatomists. It is difficult to determine whether the *bacteria actually pass out of the lymphatics* into the surrounding tissue, or whether in all such cases aberrant lymph glands are first affected. In like manner, bacteria may pass along the thoracic duct without causing any inflammation of the duct, as in Case V, above, but not infrequently, there is real infection of the duct and in rare cases, actual thrombosis. This is illustrated by Case VII.

Case VII. R.K. Age 17. History no. 57209 Autopsy no. 9396.

HISTORY Two weeks before admission, the patient was playing baseball and, while sliding to base, he scraped the skin from his left thigh. A small abscess developed, which was incised, with inadequate drainage below, and a second incision was made several days later. During this time, the glands in the groin began to swell and did not subside after the incision. Four days before admission, the patient began to have headache, pains in the back, general abdominal discomfort, and nausea. A blood culture was taken and hemolytic streptococcus was found. He was then given five doses of antistreptococcus serum, the first three at four-hour intervals, and the last two at six-hour intervals. Four days before admission, *his* temperature was *105° F. The day before* admission, the left ankle and the right sternoclavicular region became red and swollen.

PHYSICAL EXAMINATION. The patient was a well-developed and well-nourished young man, acutely ill, and in considerable pain. The wounds of the thigh showed very little discharge, and there was no evidence of healing. The glands of the left groin were enlarged. No petechiae were present. The heart action was irregular, with a systolic murmur. Breath sounds were suppressed over the *right chest* posteriorly. The temperature on admission was 102° F., the pulse rate 68, and the respirations 24. The white blood count was 20,000,



FIG. 21 CASE VII: Hemolytic streptococcus with thrombosis of the thoracic duct following abrasion of the thigh Thrombosis of thoracic duct. x = thrombus (Slightly more than life size) (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

with polymorphonuclears 91 per cent A blood culture again showed hemolytic streptococcus.

COURSE. The next day the patient became more toxic and delirious, his temperature rose to 104° F, pulse 130, and respirations 32 The chest showed further suppression of breath sounds On the third morning, he became completely irrational, his temperature mounted to 105° F., his pulse rate to 140, and his respirations to 44, and he died.

AUTOPSY. On the left thigh were two flat, ulcerated areas, which did not involve the muscles The right pleural cavity contained 200 cc of turbid fluid and the posterior part of the lung was covered with a thick, fibrinopurulent exudate. The posterior portions of the lung were compressed, and scattered throughout the lung were a few hemorrhagic areas. The left lung showed small, hemorrhagic areas without other pathology. The pericardium was nor-



Fig. 22. Case VII: Thrombosis of thoracic duct, × 15. *Insert.* Streptococci in thrombus, × 1400 (From Nelson's *Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)



Fig 23 Case VII: Peritonitis showing the relatively poor cellular infiltration, × 150. *Insert.* Streptococci in peritoneal exudate, × 1400 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 24. CASE VII: Consolidation of lung and pleural exudate, × 50 *Insert* Streptococci in pleural exudate, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

mal except for a few petechial hemorrhages. The heart showed no valvular vegetations but one of the mitral leaflets showed a petechial hemorrhage. The peritoneal cavity contained 200 cc. of thick, creamy, yellow pus, mostly in the pelvis, with the intestines bound together by a fibrinopurulent exudate. The lymph glands along the left iliac veins and artery, and those in the left inguinal region, were markedly swollen with tense capsules. They were considerably larger and softer than those on the right side On section, they were found to be edematous, and in a few places, free pus was present. Enlarged glands extended all the way up the posterior wall of the abdominal and thoracic cavities and along the thoracic duct. The thoracic duct itself was greatly distended, in places 1.5 cm in circumference. It was filled with purulent material. The receptaculum chyli was distended with pus, and the wall was perforated by a ragged hole that extended into a lymph gland. Near the upper end of the duct, the lumen was occluded with an oval thrombus just on a level with the arch of the aorta. The duct above it was filled with pus, but its lumen, was less than 2 mm in diameter. The veins of the pelvis showed no evidence of thrombosis. No gross abscesses or infarcts were visible in the spleen, liver, or kidneys Pus was found in both sternoclavicular joints Microscopically, the lymph glands and thoracic duct showed acute inflammation with many streptococci. Many of the small areas of the lungs were found to be small abscesses in which large numbers of bacteria were found, and the hemorrhagic areas proved to be small septic infarcts. The pleural exudate

showed streptococci. Bacteria were also found in the fibrinous exudate over the intestine. A few small vessels in the kidneys were plugged with masses of bacteria.

This case illustrates the entrance of organisms through the skin at an abraded area and their spread through the lymphatic glands and the thoracic duct, with the unusual result of a thrombosis of the thoracic duct at the level of the arch of the aorta. There was, further, a direct spread to the peritoneum and a venous spread probably to the lungs and through the heart to the arterial system, with localization in various tissues and organs. From the lungs, it spread directly to the pleura. The local veins were surprisingly free. The case also illustrates the inefficiency of anti-streptococcus serum in a lesion of such extent (see section on the serologic adjuncts of surgery). Had the sulfonamides or penicillin been available, this case probably would have been saved (Figs. 21-4).

IX. INFLUENCE OF TRAUMA TO OR MANIPULATION OF THE INFECTED PART IN THE SPREAD OF BACTERIA

Frequently, a patient presents himself who has a serious infection that began as a trivial pimple or boil, which was squeezed or cut, and which promptly after these manipulations became very much worse. It is assumed that in such cases the infection is spread by the trauma that causes an injury to the local tissues or produces a break in the local defensive wall of cellular infiltration or actually forces the bacteria into lymph or blood channels. This is a common clinical observation, but the sequence of events is not easy to demonstrate pathologically. A similar result may follow the injection of a local anesthetic into an inflamed area, particularly if the subsequent incision is not carried the full length of the injected area, so that the pressure caused by the injection is relieved

X. THE FATE OF BACTERIA IN THE BLOOD STREAM

In experimental work, it has been known for a long time that living bacteria could be introduced directly into the blood stream of animals, and that death depended upon the kind, virulence, and number of organisms injected. In the case of many organisms of high virulence, there is generally a fixed minimal lethal dose of several thousand organisms. For example, by frequent animal passage of hemolytic streptococci, Dochez and his co-workers[5] were able to increase the virulence of only 20 per cent of their strains to the point where 1000 organisms would kill a mouse. This has been repeatedly confirmed so that we can be sure that the animal body and probably the human body have the power to destroy

reticulo-endothelial system. The humoral components include the various elements in the blood, natural or acquired, agglutinins, opsonins, bacteriolysins, antitoxins, leucines, plakins, and complement.[7]

It may be stated as a general rule that no septicemia is maintained unless there is some focus constantly or intermittently pouring bacteria into the blood. When blood cultures are taken from patients, it must be remembered that the blood from the vein of the arm has passed through a complete circuit of the body after leaving the source of infection—that is, it has to pass through the *right heart, the lungs, the left heart, and* the general circulation, and possibly, also, the capillary circulation of the liver. The organisms that may be discharged from the original focus are, therefore, subject to the inhibiting or bactericidal substances in the blood and have run the gauntlet of two or three capillary systems, at least thirty or forty times, for every generation of the bacteria. It is evident that organisms actually discharged into the circulation frequently never reach the site from which blood cultures are taken. We often see cases with very evident metastatic abscesses in which repeated blood cultures have been negative. The greater the number of blood cultures taken, the greater will be the number of positive results in any given infection; and it is obvious that the outpouring of organisms into the circulation may not be a steady process but may be *intermittent, depending* upon many physical factors. For example, the operative procedure on a focus of infection, because of the trauma and the opening up of new channels of absorption, not infrequently causes a sudden outpouring of bacteria into the blood, and organisms may in fact be recovered by blood culture directly after the operation.[8] Martin[9] emphasized the fact that the finding of bacteria in the blood may not be of itself an indication of *grave prognosis, provided that the focus from which they come is reached* and drained, so that the organisms will tend to be discharged outside rather than inside the body.

In the greater number of surgical infections that recover, we can merely speculate with regard to the spread of bacteria from the site of infection. It is only in those cases that go on to a *fatal termination* that we can even attempt to demonstrate the manner in which the organisms spread. It is certain that in the great majority of patients who die having organisms persisting in the blood stream, it can be demonstrated that there is a thrombosis of the veins or lymphatics in the surrounding structures; or that there is a focus in the heart from which organisms are constantly being discharged into the blood stream.

XI. RELATION OF WANDERING CELLS TO BACTERIAL SPREAD

It has been repeatedly demonstrated that in the early stages of inflammation, wandering cells make their way out through the walls of the capillaries into the region of infection (Fig. 25), and that they take up the bacteria within themselves to a varying degree, according to the virulence of the organism. The positive chemotaxis that has brought the leukocytes to the point of infection now appears to lose its power, and many of the phagocytes travel in the opposite direction, away from the point of infection. In many cases, the leukocytes destroy the organisms that they have ingested, but it is very evident that in other cases, the bacteria grow and multiply within the leukocytes (see Ch. XII, Fig. 1), and may be carried by them away from the point of infection, either to the neighboring lymph glands or into the general circulation.

XII. SPREAD ALONG SECRETION OR EXCRETION PATHWAYS

A question still under discussion is the spread of organisms upward against the stream in the organs of secretion and excretion—the ducts of



FIG. 25. Showing several stages in the migration of leukocytes in a case of hemolytic streptococcus cellulitis A. Blood capillary; B Lymphatics 1, 2, 3, 4, and 5 show stages of migration. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

the salivary glands, the bile and pancreatic ducts, the ureters and tubules of the kidney, and the sexual organs both male and female. There seems to be definite evidence in the clinical progress of such infections that a spread takes place from below upward, but whether the bacteria pass up against the stream, or by a spread of the infection in the wall of the ducts, or by lymphatic or vascular channels in the deeper parts of the ducts, has not been definitely determined. The flow from these glands is not constant but intermittent, depending upon the physiologic activity of the gland. When the flow is taking place, it is obvious that organisms, as well as any particulate matter, either in the fluid or on the surface of the duct, must be carried away. However, when there is stasis, either physiologic or following obstruction, it is conceivable that bacteria may spread upward in the column of fluid just as they do in a culture medium in a test tube.

As far as the genitourinary tract is concerned, the opinion seems to be that infection does not take place when organisms are introduced through the urethra unless there is an obstruction to the outflow of urine or unless there is residual urine.[10] There may be, and frequently is, a physiologic delay, particularly after any operation around the genitals when voiding is difficult. It has generally been considered that this is largely the result of a reflex spasm of the sphincter muscle.[11] The bladder becomes dilated, and this may cause an injury to the wall. The spasm causes an obstruction, the urine remains for a longer time than usual, and the retained bacteria multiply to a corresponding degree. With children, the response to the ordinary urinary reflex is often irregular, resulting sometimes in involuntary micturition and at other times in prolonged retention of urine. This corresponds to the postoperative distension of the bladder in adults. It has been observed that when a fluid impervious to the x ray has been injected into the bladder, particularly in children, this may be carried up into the ureters by the simple act of coughing.[12] Thus bacteria may be injected from the bladder into the ureters; or, if the inflammation around the opening of the ureter causes the slightest degree of obstruction, it is conceivable that the organisms may spread upward in the column of urine. The spread by direct extension or by lymphatic or venous drainage is in every way similar to that elsewhere, and undoubtedly accounts for a certain number, if not the great majority, of infections that apparently take a retrograde course along these secretory and excretory tracts. Likewise, the infection that comes by way of the arterial stream may spread downward from the gland to the exit of the duct, and clinical cases are available indicating that this not infrequently takes place. Cases VIII and IX illustrate ascending infections, the latter from the bladder and the former from the pelvis, outside the bladder.

Case VIII. E.D. Age 56. History no. 50121. Autopsy no 9137.

HISTORY. The patient had reached the menopause twelve years previously. A year before admission, she began to have vaginal bleeding, at first, once or twice a month and then with increasing frequency. For the last three months, bleeding had been constant

PHYSICAL EXAMINATION This was negative except for a very obvious epithelioma of the cervix uteri. The blood urea was normal.

COURSE. The patient was treated by dilatation and curettage, followed by radium. Later a complete hysterectomy was performed. On the seventh day, the abdominal wound disrupted, with protrusion of the viscera. This was repaired, and the patient seemed to be in fair condition until three days later, when she suddenly had an attack of dyspnea and cyanosis, with unconsciousness. This attack passed off after a few hours, but her temperature rose to 103 6° F. and her pulse rate became 120. A similar attack occurred on the next day, and on the day following, she relapsed into a comatose condition. A test for blood urea then showed 2.55 gm per liter. The urine showed a heavy precipitate of albumin and a few pus cells. The patient became incontinent, and urine flowed from the vagina, evidently from a vesicovaginal fistula. On the thirteenth day, her temperature rose to 105.8° F. and her pulse to 128 The blood urea became 3.22 gm. per liter She died in coma on the fourteenth day after the first operation.

AUTOPSY. The thoracic cavity and pericardium were normal There was no generalized peritonitis, but the omentum and coils of intestine were adherent in the pelvis, where there was a large abscess cavity in the site formerly occupied by the uterus and tubes. The cavity was filled with pus and necrotic friable material. Both ureters passed through this necrotic wall, and their lumens were greatly compromised. The right ureter was greatly injected all along its course. The pelvis of the right kidney was slightly dilated and extremely hyperemic and injected. The right kidney was larger than the left. The stellate veins were injected, and on section, minute white flecks were seen in the kidney substance, chiefly in the medulla. There were hemorrhages at the bases of the pyramids. The cortex was of normal width The left kidney and the upper part of the left ureter were relatively normal. A small vesicovaginal fistula, 8 mm. in diameter, was found. The mucosa of both the bladder and the vagina was injected. The organs of the chest and the other organs of the abdomen were essentially negative.

Microscopically, the right kidney showed a necrotic inflammation of the pelvis with no evidence of epithelium remaining The collecting tubules were distended with blood and pus cells, and in the medulla were many small abscesses. The tubules contained many bacteria and were acutely inflamed, whereas the glomeruli were relatively normal The left kidney showed some degeneration of the tubules, but no acute inflammatory reaction. The abscess wall in the pelvis showed an extensive necrosis of the tissue loaded with bacteria of varied morphology.

This case illustrates the entrance of bacteria through the genital tract and a spread up along the ureter to the pelvis of the kidney, with a

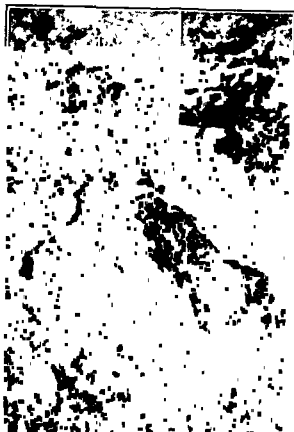


Fig 26. Case VIII: Pelvic abscess followed by pyelitis and suppurative nephritis. Pelvic abscess wall, × 150. *Insert.* Mixed bacteria in abscess, × 1500 (From Nelson's *Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)



Fig 27 Case VIII: Intense inflammation of kidney pelvis, × 15. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 28. CASE VIII: Suppurative nephritis showing tubules destroyed and containing masses of bacteria but glomeruli intact, × 300. *Insert:* Bacteria, chiefly bacilli, in tubules of kidney, × 1500. (From Nelson's *Loose-Leaf Surgery*. Courtesy of Thomas Nelson and Sons)

destruction of the tubules, but without extensive involvement of the cortex of the kidney (Figs. 26-8).

Case IX: M.K. Age 35. History no. 49605. Autopsy no. 9531.

HISTORY. For a long time, the patient had suffered from chronic nephritis, beginning with scarlet fever in childhood, acute Bright's disease at twenty-one, and recurrence with the last two pregnancies. Five months before admission, she began to have a dull pain in both kidney regions, occasionally shooting down into the groin. Two months later, pus was found in the urine. This disappeared after one month. Five days before admission, she had a chill, followed by a fever and sweating, with nausea and vomiting, which persisted. Her urine became cloudy and at times was red in color. Burning was experienced during urination. The urine had been scanty. Two days before admission, the patient had a convulsion with loss of consciousness. This was followed by repeated convulsions.

PHYSICAL EXAMINATION. The patient was a pale, prostrated, dehydrated woman. The lungs were negative. The heart was normal but its action rapid. Both kidneys, particularly the right, were large and quite tender, anteriorly and posteriorly. The right rectus muscle was spastic. The lower abdomen was tender in the midline over the bladder and on both sides, more on the right than on the left. A pelvic examination revealed considerable tenderness high



Fig. 29. Case IX: Colon bacillus cystitis, followed by pyelitis, suppurative nephritis and septicemia Bladder showing masses of bacteria in wall, × 150. *Insert*: Gram-negative bacilli in bladder wall, × 1500. (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



Fig. 30 Case IX Kidney showing extensive involvement of tubules but relatively normal glomerulus, × 150. *Insert* Gram-negative bacilli in kidney tubules, × 1500 (From Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)



FIG. 31. CASE IX: Abscess of lung, × 15 *Insert:* Mixture of bacteria in lung abscess, × 1500 (From Nelson's *Loose-Leaf Surgery.* Courtesy of Thomas Nelson and Sons)

up on the right side, but no masses were felt. The urine showed gross pus and a few granular casts, with a heavy trace of albumin A white blood count was 14,200 with polymorphonuclears 82 per cent. The temperature was 102.2° F. and the pulse rate 112. The blood pressure was 85/65.

COURSE. A blood culture yielded *E coli (communior)*. For the first two days, there was no urinary output. The patient was treated with infusions, colon irrigations, and hypodermoclyses. The urine increased somewhat, but never exceeded 675 cc. in twenty-four hours. The blood urea was 1.48 gm. per liter, gradually rising to 2.84. The blood creatinin rose from 4.5 to 7.05 mg. per 100 cc. The patient vomited daily. Her temperature gradually fell. She died on the seventh day after admission. A postoperative estimation of blood urea showed 3.54 gm. per liter and blood creatinin 9 44 mg. per 100 cc.

AUTOPSY. The body was well developed and well nourished. The heart was normal. Both lungs were adherent to the parietal pleura by old adhesions. In the right lung were several soft areas of necrosis. The abdominal organs were negative except for a contraction of the bladder. Both kidneys were red and swollen The cut surfaces were mottled with red hemorrhagic and pale yellowish areas streaked with red. The ureters and pelves were not dilated, but the mucosa was injected and they contained sanguinopurulent fluid. Microscopically, the lungs showed many pus cells in the granular necrotic masses and Gram-positive cocci in pairs, as well as Gram-negative bacilli The kidneys revealed the tubules to be markedly inflamed and filled with pus cells.

The glomeruli were, for the most part, relatively normal, but some were involved in the purulent process. Masses of Gram-negative bacilli were present in the tubules. The epithelium of the bladder had disappeared. On the surface were large clumps of Gram-negative bacilli. The other organs were relatively normal.

This case illustrates an infection with *E. coli.* The pathologic evidence suggests a primary infection of the bladder, a spread to the kidneys, where the tubules were largely involved and the glomeruli relatively free, and, subsequently, a bacteremia. The lung abscesses showed a mixture of organisms, some of which probably came from the throat, getting a foothold in an area already involved by *E. coli* (Figs. 29-31).

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CHAPTER XIV

Bacterial Synergism

SYMBIOSIS SIGNIFIES the living together of two different species either of plants or of animals, or of a plant with an animal. There is fossil evidence that this is a very ancient phenomenon of nature, and many believe that it is the most potent factor in the origin of species. Wallin,[1] in his book on symbionticism, uses the term 'proteotaxis' to describe the innate tendency of one organism or cell to react in a definite manner to another organism or cell. This may be positive or negative, and positive proteotaxis may result either in parasitism or symbiosis. Parasitism implies some combination in which the parasite benefits while there is either a harmful or an indifferent response, or sometimes death on the part of the host. The term *symbiosis* is applied to those cases in which mutual benefit is derived by the associated organisms. Steche[2] considers that parasitism and symbiosis are similar relationships. In the former, one cell lives at the expense of the other and in the latter there is a balanced condition not injurious to either and usually beneficial to both. Parasitism is a battle, symbiosis an armistice or an alliance. In symbiosis each partner uses the other. It is usually a matter of trade—with each one giving something of no value to itself and receiving something of great value to itself. If at any time one of the two organisms cannot fulfil the necessities of the other, a parasitic battle ensues, which usually results first in the death of the weaker and then in the death of the stronger. Under other conditions, parasitic states may gradually change to symbiotic states. On this basis Rheins[3] suggests that in many cases in which individuals have recovered from symptoms of tuberculosis, they are really living in symbiotic relationship with the tubercle bacillus. On the other hand, Rheinheimer,[4] although he holds to the importance of symbiosis in the processes of evolution, believes that parasitism and symbiosis are at opposite poles. To quote him:

> The symbiotic relation is characterized by reciprocal differentiations of a progressive order; by due compensation as between the partners; by widely availing usefulness in the web of life. Parasitism, on the other hand, is the denial of such reciprocity, the undoing of its good effects. It is the antithesis of symbiosis, as the facts bear out abundantly when they are stripped of mis-

leading details. Symbiosis is a good relation; parasitism is an evil one. Good and evil, of course, are relative terms. But once we have a standard by which reliably to judge biological activities and their results, we need not shrink from the attempt to discriminate between legitimate and illegitimate developments, and thus to introduce order into biology in the place of the present chaos and indeterminateness of jargon. The claim made by some that biology is not a matter of values is preposterous. It is a species of scientific fatalism no longer warranted today. The very term 'survival of the fittest' involves a value-judgment. There is no cause for fatalism or pessimism on a due appreciation of symbiosis, no cause to falter and to think that Nature sanctions crime equally with good conduct. True, in the course of evolution, legions of species have strayed from the symbiotic path and have elected instead that of least resistance—that of predacity or of parasitism. But it can be shown that they have declined accordingly, whilst others, which have persisted in a more honest course, have meanwhile gone forward.

On this basis, Rheinheimer offers a new theory of disease concurrently with that of symbiosis. He says: 'Disease, degeneration and extinction originate with failure of co-operation, be it between organs or species.' Wheeler [5] states in an article on 'Social Life among the Insects' that 'Living beings not only struggle and compete with one another for food, mates and safety, but they also work together to insure to one another these same indispensable conditions for development and survival.' Hastings [6] says that 'Life is not only a struggle for existence among half-starved individuals thirsting for each other's blood but plants and animals of different kinds cooperate to a much larger extent than was formerly supposed.'

One of the best examples of symbiotic existence is the lichen, which is formed by the intimate association of a fungus and an alga. Lichens perform an important function in serving as the advance guard of vegetation by reaching out over the desert and into the cold of the north and by climbing mountains to the snow line. They cover bare regions of land, and are often followed by other vegetation. A still more important function is performed by nitrifying bacteria, such as *Bacillus radicicola*, which occur in the soil. These, by entering the root hairs of leguminous plants and growing within the roots, take up nitrogen from the air and supply the roots with nitrates, accepting in return carbohydrate for their own nourishment. Bacterial mixtures play an extremely important function in the disintegration of animal bodies. Many other instances of these relationships could be cited, but these will serve to indicate the importance of the general laws of symbiosis without which life as it exists on the earth would cease.

Since the early days of bacteriology, it has been observed that different species of bacteria frequently exist together, but very few reports have

been made with regard to the effects that these organisms have on one another. Since the time of Koch,[7] much greater importance than formerly has been placed upon obtaining organisms in pure culture. There is very little evidence in the literature that, even during World War I, when wound infections with mixtures of organisms were commonly observed, much thought was given to the symbiosis of these organisms. In the intestine of man and of animals, bacteria have symbiotic relationships some of which are mutually beneficial, whereas others are antagonistic. It is still a moot question whether or not this state of affairs is beneficial to the host.

The symbiosis of bacteria has been observed in the laboratory for a long time. Certain species of bacteria completely inhibit the growth of other species. Certain species have an adjuvant action on the growth of other species. Still other species combine and perform certain functions which neither of them can perform alone. This combined activity has been called 'synergism.'

I. LABORATORY STUDIES OF BACTERIAL SYNERGISM

Most of the observations that have been made with respect to bacterial synergism have occurred by chance in the laboratory and have had no clinical importance. Pasteur[8] found that anaerobes could grow in the presence of free oxygen provided that aerobes were present, and he explained this phenomenon by suggesting that the aerobes used up all of the available oxygen. McLeod[9] has tried to explain this symbiotic relationship by demonstrating that the anaerobes are not able to produce peroxidase enough to destroy the peroxides that are formed when growth takes place in the presence of free oxygen, but if aerobes are present *they* furnish the catalase that destroys or neutralizes the peroxide that would otherwise kill the organisms. Herter,[10] in his book on the infections of the digestive tract, states that were it not for this symbiotic action of anaerobes with aerobes, the former could exist only in the large intestine, which is without oxygen, for oxygen is present in the small gut.

Some of these observations are of considerable importance commercially. Ward[11] noted that certain fermentations in ginger beer were caused by a combination of a yeast with the bacterium. Nencki[12] showed that the bacillus of symptomatic anthrax and a micrococcus together produced butyl alcohol from a fermentable carbohydrate, when neither of them could do it alone. Castellani[13-15] has been interested in the subject of bacterial synergism for some time. His first observations were made in a study of bakers' yeast in Ceylon When he separated the many species of organisms in the yeast, he found that his pure cultures would not produce gas; but when certain combinations were made, gas was

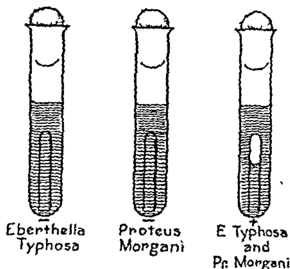


FIG. 1. The synergistic action of two organisms producing gas from maltose, a function neither could perform alone (Adapted from Castellani [15])

formed. Castellani later carried his observations to pathogenic bacteria and found that gas was formed in media containing certain of the complex carbohydrates by the synergism of two species while the pure cultures of the individual organisms failed to produce it. Thus *Eberthella typhosa* with *Proteus morgani* produced gas with maltose, and similar effects were obtained with dysentery bacilli and certain other organisms, which were of differential importance. When these facts had been determined, it was possible by such reactions to determine the carbohydrate present if the organisms were known, or the organisms present if the carbohydrates were known.

Castellani agrees with other observers in assuming that in such a symbiosis, one organism initiates the process while the other completes it. In the case of fermentation, he believes that one organism forms acid from the carbohydrate and the other produces gas from the acid. Holman and Meekison [16] made the same observations and came to the same conclusions. They demonstrated that in order to perform this function, the two microbes must be actually growing together in intimate association, and that it was not the effect of one bacterium on the other. Sears and Putnam [17] went into this matter of gas production a little more fully and found that bacteria could be divided into three groups: the nonfermenters, the acid formers, and the gas formers. In this particular kind of synergism the nonfermenters never take part. It is always the result of a combination of an acid former with a gas former. The degradation of the sugar in question is begun by the acid former. In the course of the decomposition, substances are formed that are utilizable by the gas former, and gas production results. Sears and Putnam repeatedly failed

in the attempts to produce gas by inoculating gas-forming organisms into a medium in which an acid former had been grown but which had subsequently been sterilized either by filtration or heat, so that they believed that the second organism did not act on the end product of the first but upon some intermediate product. Sherman and Shaw [18] demonstrated the synergism of two organisms in the production of propionic acid. *Bacillus acidi propionici*, which is the essential organism for the production of 'eyes' and the characteristic flavor of Swiss cheese, will produce a very much larger amount of propionic acid in association with several other organisms than it will alone in a medium containing lactose. The associated organisms may be either lactose fermenters or nonlactose fermenters. Ishikawa [19] made some very interesting observations on the synergistic action of certain bacteria. He combined not two but three different species. He confirmed the findings of others that an acid former and a gas former would together produce gas from a complex carbohydrate, but if nitrogenous substances were added to the carbohydrate medium, the two organisms that ordinarily in symbiosis would break down the carbohydrate to form gas would not do so unless there were also present a proteolytic organism to initiate the breaking down of the proteins present. The products of protein digestion favor the activity of amylolytic enzymes. Thus, we have a synergistic action requiring the presence of three different kinds of organisms, but in this instance, the activity of the proteolytic organism takes place *before* and not necessarily *with* the activity of the other organisms. Theobald and Dorothea Smith [20] have shown that just as there is synergism with certain organisms in the production of gas, there is a corresponding antagonism with other organisms. They observed that bacteria of the paratyphoid group may be divided into two classes according to the behavior of four-day cultures in lactose bouillon after a second inoculation with certain types of *E. coli*. *Escherichia coli* produces gas after true hog-cholera bacilli have grown in the medium but produces no gas after other paratyphoid bacilli. Likewise Speakman and Phillips [21] found that characteristic production of large amounts of lactic acid by the association of *B. granulobacter-pectinovorum* was prevented by some factor produced in *B. volutans* cultures. *Bacillus granulobacter-pectinovorum* usually carries the fermentation down to acetone and butyl alcohol but it stops with lactic acid if *B volutans* is present. Burri and Stutzer [22] found that two organisms when combined would produce nitrogen gas from nitrates when neither would do it alone. They showed that one reduced the nitrate to a nitrite and the other produced free nitrogen from the nitrite.

In our study of the organisms in raw catgut, we found two instances in which a combination of the organisms present in a single specimen of catgut produced a lethal effect when injected into an animal, whereas

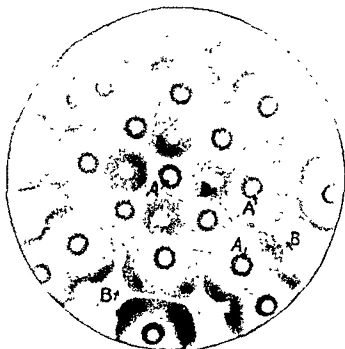


FIG 2. The synergistic action of two aerobic organisms made visible on a blood-agar plate. When the colonies of a nonhemolytic diphtheroid are in close proximity to the outer zone of a double-zoned staphylococcus, some substance emanating out from the diphtheroid colony completes the lysis of the outer zone of the staphylococcus

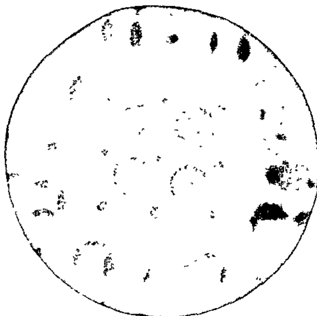
the organisms in pure culture failed to do so In two other instances, abscess and skin necrosis were similarly obtained by bacterial mixtures. Furthermore, in two specimens of *improperly sterilized* catgut, a mixture of organisms was found that had a very prompt lethal effect on laboratory animals when injected together but that were entirely nonpathogenic when in pure culture.[23]

Some striking examples of synergistic action in the production of hemolysis on blood-agar plates[24] have been observed in the author's laboratory. The phenomenon was noted with three different groups of organisms: a pair of aerobes, a pair of anaerobes, and an aerobe with an anaerobe. In the exudate from a case of chronic empyema of tubercular origin, we found, among other organisms, a double-zoned *Staphylococcus aureus* and a diphtheroid bacillus. On blood-agar plates, the colony of the double-zoned staphylococcus has a narrow zone of clear hemolysis immediately around it and a wide zone of partial hemolysis about 8 to 10 times the diameter of the colony. It so happened that when the colonies were fished from the original blood-agar culture to a fresh plate the diphtheroid bacillus and the double-zoned staphylococcus were streaked side by side. After incubation, this plate showed that on the side toward the diphtheroid bacillus the outer zone of the staphylococcus



FIG 3 The synergistic action of an aerobe and an anaerobe made visible on a blood-agar plate The colony of a hemolytic *B. subtilis* exerts a hemolyzing influence on the outer zone of *C. welchii*

FIG. 4 The synergistic action of two anaerobes made visible on a blood-agar plate. The colony of *C. sordellii* has a hemolyzing influence on the outer zone of *C. welchii*



colonies was completely hemolyzed over an area very evidently under the *influence of some diffusable substance* or physical force emanating from the colonies of the diphtheroid bacillus. Immediately around the diphtheroid colonies no change in the red cells was visible.

In order that this effect might be brought out more clearly, a design was made on another plate by alternately dotting with the two cultures. Photographs of the plates show the effect produced by the two organisms when in juxtaposition (Fig. 2). It was found that control nonhemolytic colonies of several other species did not have this effect, but the diphtheroid bacillus had the same effect on the outer zone of both hemolytic and nonhemolytic *C. welchii*. If the staphylococcus was planted alone and incubated for twenty-four hours, and the bacillus was subsequently planted on the same plate, the same hemolysis of the outer zone took place. Some months later, when a specimen of improperly prepared surgical catgut was cultured, two anaerobic organisms were found that had exactly the same relationship to each other as had the *Staphylococcus aureus* and the diphtheroid bacillus. They were a double-zoned hemolytic strain of *C. welchii* and a nonhemolytic strain of *C. sordellii*. The plate after anaerobic incubation gave the same appearance as the two aerobic organisms gave before (Fig. 3).

Later, a third example of the same phenomenon was observed when, from another specimen of catgut, a hemolytic strain of *C. welchii* and a hemolytic strain of *B. subtilis* were found. The *B. subtilis* colony had a narrow zone of clear hemolysis about it and an outer zone of influence not visible until it came in contact with the outer zone of the *hemolytic C. welchii* colony which it completely hemolyzed (see Fig. 4).

II. CLINICAL EXAMPLES OF BACTERIAL SYNERGISM

There are very few proved instances of disease processes due to the synergism of two species of bacteria. Castellani[15] believed that a good many symptoms in certain diseases that are ascribed to the causative organism are really due to the association of symbiotic organisms. Among these, he included excessive tympanites with typhoid fever. He lists three definite disease entities attributable to the synergistic action of two organisms. *Trichomycosis nigra*, a disease of the hair of the axillary and pubic regions, is caused by a fungus *Nocardia tenuis* and a coccus [illegible]

organisms, one a fungus and the other a bacillus. Vincent's [illegible] been considered a disease of symbiotic organisms, a fusobacterium and a *spirillum*. Some authors have believed them to be morphologic varia-

tions of the same organism. Knorr,[25] who has made some interesting laboratory studies with regard to this disease, believes them to be different organisms living in symbiosis, but he has shown that in conjunction with some of the mouth streptococci they perform certain functions that they cannot perform when separated from the streptococci. When cultures are made from the mouth under a seal, the streptococci predominate at first, then the fusobacteria, then the spirilla, and finally the spirochetes. Knorr believes that this shows that one organism prepares the ground for the growth of the others and that infection in the mouth with the fusobacterium and spirillum occurs only following a preliminary infection with streptococcus. Roux and Vaillard [26] noted that avirulent tetanus organisms became virulent when they were mixed with certain other nonpathogenic organisms, such as *Proteus vulgaris*. Novy [27] also noted the enhancement of virulence of his bacterium of malignant edema when it was mixed with nonpathogenic aerobes. Liermann [28] interested himself in the study of bacterial mixtures in certain putrefactive processes. In one such lesion, he found nine different species. When he inoculated pure cultures into animals he obtained no result. Likewise, when he injected mixtures of the organisms he produced no lesion. He then inoculated media containing sterile meat with a mixture of the organisms and injected this culture into animals, with resulting death. Here the synergistic action was exerted upon the dead meat, and toxic substances were formed that were lethal. Death was not a result of the synergistic action of the organisms on the living animal. Liermann thought that the quantitative relation of the symbionts was important for the production of the effect, but other workers have not agreed that such is the case with respect to other synergistic functions. Bienstock [29] observed that when protein is digested, the foulest odor is given off when it reaches a homogeneous mass This reaction is best accomplished by the combined activity of aerobes and anaerobes. Strict anaerobes are necessary for the ultimate changes, but aerobes greatly aid in the early stages of the process. Kammerer [30] studied intestinal organisms from the point of view of the formation of urobilin from bilirubin. He found that pure cultures of the different strains could not produce it alone. Certain mixtures were necessary, and the result was a true synergistic effect. Kämmerer also studied the production of hematoporphyrin from hemoglobin. He found porphyrin in the fluid from lung abscesses, and when he cultured the organisms present, he found that they were able to produce hematoporphyrin from hemoglobin only when they were in symbiosis.

Weinberg [31] has stressed the role of bacterial synergism in the etiology of acute appendicitis and has shown that certain combinations of the intestinal organisms are lethal in smaller doses than are pure cultures of

the organisms themselves. Our own experience in laboratory studies of peritonitis clearly confirm these findings.[32]

A. PROGRESSIVE BACTERIAL SYNERGISTIC GANGRENE, A CLINICAL AND BACTERIOLOGICAL ENTITY

1. *Clinical Manifestations*

In 1926, Dr. George E. Brewer and the author[33] reported a case of progressive gangrene of the abdominal wall following the drainage of an appendiceal abscess. The gangrene developed on the 10th postoperative day in the upper half of the wound in the neighborhood of two retention sutures of silkworm gut. The lesion was characterized by an advancing zone of redness and swelling, merging centrally into a purplish zone and then frank gangrene of the skin and subcutaneous tissue. The lesion was extremely painful. The gangrene progressed slowly but steadily in spite of conservative methods of treatment, which included opening the wound widely, the application of various antiseptics, and the local removal of the gangrenous margin. It was controlled only after a very wide excision of the whole lesion, the incision being made in normal skin 3 to 4 cm. beyond the outer margin of the red zone. A bacteriologic study of the lesion revealed the presence of a microaerophilic nonhemolytic streptococcus in pure culture in the periphery. This streptococcus was obtained only by anaerobic culture, but after nine subcultures, it began to grow sparingly aerobically. In the gangrenous margin, the organism was associated with a hemolytic *Staphylococcus aureus* and a diphtheroid bacillus. A series of experiments in animals (discussed below) demonstrated clearly that the association of the microaerophilic streptococcus and the *Staphylococcus aureus* was responsible for the lesion and that it was an example of bacterial symbiosis or synergism.

2. *Laboratory Confirmation of the Bacterial Synergism*

A. ANIMAL INOCULATION

Two cubic centimeters of a 20-hour culture of this streptococcus in cooked meat medium with 0.2 per cent dextrose injected into the peritoneum of a mouse failed to kill. Five cubic centimeters in the peritoneum of a guinea pig failed to make it ill, but in one instance the organism was recovered from the peritoneum after 2 weeks, and in another, a month later. When the ninth subculture mentioned above had acquired the faculty of growing on aerobic plates, it was injected into the peritoneum of a guinea pig. It was recovered after 24 hours and was then found to have lost the faculty for aerobic growth. Five cubic centimeters injected subcutaneously into guinea pigs and rabbits produced slight redness and swelling, which appeared on the day after injection,



FIG. 5. The lesions in a guinea pig Anteriorly (at X) 2 cc of streptococcus culture were injected Posteriorly (at O) 2 cc. of staphylococcus culture were injected without effect. In the center 1 cc. of each was injected with extensive gangrene The slough has separated—sixth day

but rapidly subsided There was never any evidence of gangrene. These failures to demonstrate pathogenicity for animals seemed to controvert the very definite evidence of activity in the tissue of the patient. It was then suggested that the effect should be tried of this organism combined with the hemolytic *Staphylococcus aureus* and the diphtheroid bacillus with which it was found associated in the actual gangrenous margin of the wound. Two cubic centimeters of a 20-hour culture of each organism were injected subcutaneously into control guinea pigs; and into another pig 1 cc. of the hemolytic *Staphylococcus aureus* was combined with the same quantity of the nonhemolytic microaerophilic streptococcus. In still another pig the streptococcus was combined with the diphtheroid bacillus In 24 hours the staphylococcus combined with the streptococcus had produced a large, red, tender swelling 2 x 3 cm. in diameter with a central area of discoloration indicating beginning gangrene. The staphylococcus alone produced a somewhat smaller, red, tender swelling, without any evidence of gangrene. The streptococcus alone produced only a slight red swelling, as did the combination of streptococcus and diphtheroid bacillus. The diphtheroid bacillus alone produced no lesion.

In the second 24 hours, the lesion with the staphylococcus alone increased slightly in size but thereafter subsided, finally localizing as a small abscess, from which the organism was recovered after 2 weeks. The lesion produced by the combination of staphylococcus and streptococcus increased in the second 24 hours and a large irregular area of



FIG. 6. Another dog on the fourth day after injection, in which the mixture (center) caused an extensive gangrene and the staphylococcus alone produced an abscess (O). The streptococcus alone (X) was without effect (*From Annals of Surgery*, Vol. 94, pp. 961-81, December 1931)

frank gangrene developed, which after 5 days separated at the margin and sloughed out. Both organisms were recovered from the lesion. This *experiment was repeated twelve times in guinea pigs and rabbits, and* in every case but one produced a large lesion with more or less gangrene. The lesion in one of the guinea pigs is shown in Figure 5. In one rabbit *the mixture produced no gangrene but an abscess formed three times the* size of the controls. In one instance, a silkworm-gut suture contaminated with the staphylococcus was passed through an area into which the *streptococcus had been injected, and it was tied with moderate tension.* Gangrene developed around the suture with a wide zone of swelling outside it. The process generally reached its peak in three to four days, *and thereafter subsided. It did not spread progressively as in the human* case. The experiment was repeated in a dog in order to use an animal with thicker skin and more subcutaneous tissue. On the right side, the *staphylococcus was injected alone, on the left side the streptococcus* alone, and in the center the same quantity of these two, mixed together.

A total volume of 5 cc. was injected into each area, the pure cultures being diluted with broth. Care was taken to put each injection into the subcutaneous tissue with a minimum of trauma at the point of injection. In 24 hours, a moderate swelling appeared at the site of the staphylococcus injection and a slightly larger one at the site of the combination Only slight swelling appeared where the streptococcus was injected alone. In four days the mixture had produced a large swelling four times as large as the staphylococcus alone and there was an irregular patch of early gangrene in the center. The staphylococcus lesion thereafter subsided without gangrene.

On the fifth day, frank gangrene developed at the injection site of the mixture and on the sixth day it sloughed out, leaving an undermined gangrenous margin. The gangrene spread slightly for a day or two and then subsided, but showed very little tendency to heal (Fig. 6).

3. *Another Clinical Example*

Five years later, a man of 35 came to the Presbyterian Hospital complaining of a diffuse abdominal pain of two weeks' duration. On examination, a mass could be felt by the rectum. At operation an abscess was found in the pelvis containing 30 to 40 milliliters of thick, green pus. The abscess was drained by means of two cigarette drains and a large rubber tube. Two silkworm-gut retention sutures were placed in the upper part of the wound. Cultures from the exudate yielded *Escherichia coli*, *C. welchii*, and a nonhemolytic microaerophilic streptococcus. On the third day, it was obvious that the wound was infected. On the eleventh postoperative day, it was observed that there was infection around the retention sutures and on the thirteenth day it was noted that the wound had a 'carbuncular appearance' in the upper half. The lesion showed such a striking resemblance to the previous case of Dr. Brewer's that when the author saw the case he advised wide excision of the lesion and prompt application of antiseptic fluids. This resulted in a prompt disappearance of the infection, the denuded area granulated over rapidly, and on the eleventh day following this second operation, the wound was covered with Thiersch grafts, which took nicely.

4. *Further Laboratory Evidence*

The excised plaques of skin and subcutaneous tissue were transferred to the laboratory for examination. Two separate lesions were available for study. In each case, the periphery of the lesion yielded the same microaerophilic nonhemolytic streptococcus in pure culture (Fig. 7), whereas the gangrenous portion yielded this organism in conjunction with a faintly hemolytic *Staphylococcus aureus* (Fig 8). *Clostridium welchii* and *E. coli*, which had been present with the streptococcus

Fig 7. Pure culture of the microaerophilic nonhemolytic streptococcus from the periphery of the lesions cultured anaerobically on a blood-agar plate. (From *Annals of Surgery*, Vol. 94, pp 961-81, December 1931)

Fig. 8. Mixed culture of the microaerophilic nonhemolytic streptococcus and the *Staphylococcus aureus* from the gangrenous margin. The plate was incubated for 24 hours anaerobically to permit the growth of the streptococcus (pin-point colonies), and then for 24 hours aerobically to permit further growth of the staphylococcus (large colonies). (From *Annals of Surgery*, Vol. 94, pp. 961-81, December 1931)



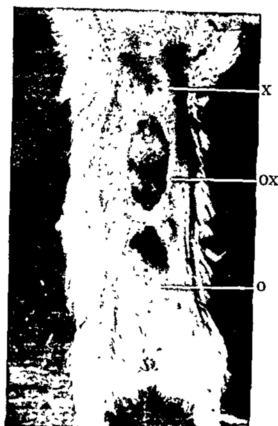


FIG 9. A lesion in a guinea pig, with the area of the staphylococcus injection (O) approximating the area of the mixed culture injection (OX) In this region there is a semi-lunar arc of necrosis. The area of the streptococcus injection (X) is unaffected. (From *Annals of Surgery*, Vol. 94, pp. 961-81, December 1931)

organisms and the control injections of the pure cultures are in close proximity, the margin of the area receiving the staphylococcus injection toward the central lesion will take on the gangrenous appearance, but the corresponding margin of the area receiving the streptococcus will not be affected. This effect is shown in Figure 9. It may be that in the combined lesion, the streptococci spread more widely than the staphylococci and *reach the site of the staphylococcus injection in sufficient concentration* to produce the lesion. Or it may be that it takes a smaller dose of the streptococcus to activate the staphylococcus than vice versa.

When doses adequate to produce the lesion in the skin were injected into the peritoneum of guinea pigs, there was no evidence of disease. The peritoneum was able to take care of large numbers of bacteria in a free broth culture, but when the culture was incorporated with barium in a cylinder of agar, the combination of the streptococcus and staphylococcus caused a progressive loss of weight, resulting in death in four days. The staphylococcus alone with a double dose resulted in death two days more than the combination but without showing any illness the day before death. The streptococcus alone produced no ill effects whatsoever.

The fact that this type of streptococcus is so frequently found in lung abscesses led us to attempt to produce this lesion in animals. A suspension

of equal parts of barium sulfate and 2 per cent melted agar was sterilized by boiling and then cooled to 40° C. This suspension was poured into three small bottles and the cultures of streptococcus and staphylococcus were then added separately and together. The mixture of staphylococcus and streptococcus contained half the number of each organism used in the pure cultures. After the cultures were thoroughly mixed with the barium and agar, the mixture was allowed to solidify. With a large syringe needle a cylinder was then cut about 25 mm. in length and 1.5 mm. in diameter. To ascertain the presence of the plug in the needle, it was first expelled into a sterile dish and then sucked up into the needle again. With a small amount of saline in the syringe these plugs were then injected into the jugular veins of three rabbits. X rays showed that although they broke up to some extent in passing through the heart they generally were caught in one or both lower lobes. X rays of the lungs were then taken at intervals of two to three days. Five days after injection, the rabbit that received the mixture of organisms began to lose weight and the x-ray films showed an infiltration of the lungs around the infected emboli. This rabbit continued to lose weight while the others appeared normal. Later x rays showed progression of the lesion in both lungs. After fifteen days, however, the x ray of the rabbit receiving staphylococcus alone showed infiltration around the emboli. The animals were then sacrificed and the lungs were removed All three showed adhesions of the lung to the diaphragm in the region of the emboli The streptococcus embolus produced only an infarct. The staphylococcus alone produced a pneumonitis involving the lower third of both lungs. The combination of organisms resulted in a much more extensive involvement—at least three-quarters of both lungs being consolidated. These experiments suggest that in the lungs also these organisms have some adjuvant action upon each other (Figs. 10a, b).

In 1933, 3 more cases of this kind were reported by the author. In each of them, the microaerophilic nonhemolytic streptococcus was found in the advancing periphery of the lesion and the secondary organisms in the gangrene. Since that time, the author has seen 12 others, making 17 in all. Six of the 12 were patients in the Presbyterian Hospital and 6 in neighboring hospitals. In every one of these cases, the bacterial synergistic etiology was confirmed, and there can be no further doubt about it, although such skepticism was recently expressed by one writer.[36]

Among these cases, one deserves special mention because of its extent and the last 3 because of their response to penicillin The penicillin-treated cases have been reported.[37] The extensive lesion developed in the skin around the drainage tract of a perinephritic abscess and fecal fistula originating from a perforated carcinoma of the splenic flexure of the colon. The process extended from the spine to the umbilicus and



FIG 10. Direct photograph (A) and X-ray films (B) of the lungs of the rabbits (after removal) into which the infected barium and agar emboli were injected 25 days previously: P—streptococcus alone—very small lesion in one lung. F—staphylococcus alone—moderate lesions in both lungs PF—symbiosis with half the dose of each organism—extensive lesions in both lungs. (From *Annals of Surgery*, Vol. 94, pp 961-81, December 1931)



from the ribs to the hip. The patient had been in another hospital for one month and was transferred to the author's service when she had reached that condition. The new growth of the bowel had not been suspected but it was confirmed by biopsy. The serum proteins had fallen to 4.2 and she had a generalized edema. The lesion was widely excised but all attempts to control the hypoproteinemia failed and she died on the tenth postoperative day.

B. GANGRENOUS IMPETIGO (ECTHYMA)

For a good many years, reports have appeared in the literature describing a chronic gangrenous disease of the skin appearing in undernourished individuals both young and old who were generally in a low state of nutrition and were frequently suffering from recurrent attacks of dysentery. It has been given many names by the dermatologists, among them, ecthyma, pyoderma gangrenosum, impetigo gangrenosum, dermatitis gangrenosa, and so on. Recently such cases have been described in the periodical literature by Hartzell,[38] Hitschmann and Kreibich,[39] Wende and Bentz,[40] Hu,[41] Brunsting, Goeckerman, and O'Leary,[42] Morrissey and Reynolds,[43] McCarthy and Fields,[44] Montgomery,[45] and others. Most of the textbooks, Unna,[46] Stelwagon and Gaskill,[47] Sutton,[48] McLeod,[49] Fox,[50] and Andrews,[51] have stated that this disease is simply a severer and deeper form of impetigo, but Shamberg[52] was of the opinion that they were distinct.

The lesions are usually multiple, and show various stages of development, one lesion following another in rather rapid succession. They occur most frequently on the scalp, face, and abdomen, but may be found on any part of the body. They generally start as small vesicles surrounded by a red zone. The center then becomes dark and gangrenous and depressed. The lesion increases in size slightly, and occasionally two or three neighboring lesions may coalesce, but even the coalesced lesions seldom measure more than 1 or 2 cm. in diameter. The disease is contagious and frequently occurs in several members of a family at the same time. Likewise, the patient inoculates other areas of his body. As new lesions develop, the old ones frequently dry, the necrotic skin comes off as a scab, and a scar is left behind. The larger and deeper lesions, however, may persist for a long time. The gangrenous center then separates at the margin, leaving a ring of depressed ulceration, from the center of which the gangrenous plaque stands up like a button. If this separates from its base, a clean ulcer is left, which slowly heals. This condition may last for months or years with exacerbations and remissions, and frequently a crop of small fresh gangrenous lesions may develop after a recurrence of diarrhea or colitis.

The cause of these lesions has been variously explained by different



FIG. 11. Gangrenous impetigo. The lesions are usually multiple. Each lesion starts as a vesicle and then a gangrenous button forms. It is due to the synergistic action of both a hemolytic streptococcus and a hemolytic *Staphylococcus aureus* (Courtesy of Dr. William Friebos, *The Atlas of Skin Diseases*, 4 vols, Leipzig, 1930)

authors. Most dermatologists consider the condition to be a serious form of impetigo. The onset of the lesion with vesiculation makes this theory seem plausible. The organism generally attributed to be the cause of impetigo is the hemolytic streptococcus. Some authors have attributed ecthyma to the action of *Pseudomonas pyocyanea*, others to staphylococci, and still others to other streptococci. One of these echthyma cases died with a general sepsis in which three different organisms were successively found in the blood.[53] This finding leads to some uncertainty with regard to their role in the etiology of the disease. Leloir in 1880[54] found both staphylococci and streptococci in five of these echthyma cases, but he

the prior observations of Plaut. In 1905, Plaut and Vincent debated the question. In 1896, Vincent [57] had observed spirochetes in cases of hospital gangrene and believed them to be responsible for it. Eggers,[58] in 1915, in the course of a morphological study of over 2000 smears from chronic skin ulcers in patients in various parts of China, found spirochetes in about 10 per cent. They were frequently found in the lesions commonly known as 'tropical sore.' Plaut [59] later found the combination of fusiform bacilli and spirochetes in two 'nomalike' lesions of the skin.

Stiles and Hassall,[60] in their classification of the protozoa reported for man, state that the spirochetes pathogenic for man are limited to the Treponema and Leptospira groups. Smith,[61] in his recent book on fusospirochetal diseases, lists four species of spirochetes and three types of fusiform bacilli that occur in the human mouth and that have been found in human infections.

Hospital gangrene practically disappeared when surgeons began to have a little understanding of bacterial contagion and infection. It is almost certain, therefore, that it was an infectious type of gangrene. Whether or not it was due to a specific organism, to a specific combination of organisms not including the spirochetes, or to the fusospirochetal group as Vincent claimed, may never be determined. Warren [62] believed that the term included a number of different acute and subacute forms of infectious gangrene. The author cannot add anything to the solution of this problem from personal experience but agrees with Warren after studying a number of the reports that appeared in the medical literature immediately following the Civil War, and later. (See Chap. 1.)

In recent years, gangrene of the skin and subcutaneous tissues, in which the fusospirochetal organisms play a part, has generally occurred following the implantation of mouth organisms into the tissues by human bites. Flick [63] reviewed the literature in 1932 and added five cases to the ones that had been previously reported by Hultgen;[64] Peters;[65] Hennessy, Madras, and Fletcher;[66] McMaster;[67] Barnes and Bibby;[68] Pilot and Meyer;[69] Fuller and Cottrell;[70] Bower and Lang.[71]

A human bite invariably contaminates the wound with a mixture of organisms. Bates says that the police surgeons fear the severity of these infections more than any other type of infected wound.[72] Usually gangrene does not develop in these cases unless there is a mixture of organisms including nonhemolytic streptococci, fusiform bacilli, and spirochetes. Although spirochetes are never found alone in these infections, the worst cases are certainly those in which spirochetes are present. The infection almost always occurs either when a human being bites another or strikes a blow with the hand which is cut by the teeth of the intended victim (Fig. 12). The wound is usually a lacerated wound of considerable depth, but cases have been reported in which the injury



FIG. 12. Human bite. A blow with the left hand on the other fellow's mouth resulted in a cut over the metacarpophalangeal joint of the thumb A rapid infection developed, owing to the synergistic action of anaerobic streptococci, spirochetes, and fusiform bacilli.

was very superficial. One case followed the picking of a superficial blister with a penknife with which the patient was accustomed to pick his teeth It is surprising that more wicked infections do not develop in wounds that have been sucked, since sucking wounds is a common practice. It is probable that organisms are not planted in the depths by this procedure

Usually within the first two or three days after receipt of the injury there is some evidence of inflammation, and it steadily progresses. The exudate becomes foul, the margins of the wound are shaggy, bleed easily, and take on a dark gray-green appearance. The infection may spread fairly rapidly into the neighboring bones and joints. It burrows down into the deep spaces and may work up again toward the surface and break out at some distance from the original wound. Thus multiple sinuses are produced or if incisions are made in various places, the wounds remain open and continue to discharge the foul-smelling exudate. Unless the proper treatment is instituted, these infections go on steadily for weeks or months with a progressive destruction, not only of the skin and subcutaneous tissue, but of the deeper structures as well.

If smears are made from the foul exudate, countless organisms are seen, among them streptococci, fusiform bacilli, and spirochetes—the latter are better seen with the dark-field illumination. These organisms will develop only under anaerobic conditions and are grown best in or on special media. A great many serious human bites occur in which only streptococci and staphylococci are found, but usually in these cases, although infection is severe and the general symptoms marked, there is no gan-

Meleney's second case.[34] In amebic lesions that have no connection with a deep focus, Engman and Heithaus[66] say that the infection with amebae apparently must be preceded by an established infection with other organisms. This at once suggests the possibility of a symbiotic rather than a specific action. In such a case the infection remains relatively superficial. The spread seems to be in the cutis while the epidermis is involved secondarily and gives way. Glairy pus in small droplets may be expressed from the margin of the ulceration. This is said to be quite characteristic of the infection. If the proper treatment is not instituted, the lesion continues to spread fairly rapidly in all directions until large areas are involved and the patient finally succumbs either to the involvement of the lungs or the liver or to an intercurrent infection.

In none of the cases attributed to amebae and reviewed by the author have careful anaerobic as well as aerobic bacteriological studies been made. The bacterial factor either alone or in symbiosis with the amebae may not have been given the attention it deserves. It seems to the author that one or all of the following conditions should obtain before it can be fairly stated that amebae are participating actively in any infection: (1) There should be histological evidence of the invasion of the tissues by the amebae, (2) they should be found either by smear or culture in the advancing margin of the lesion, or (3) the lesion should respond to emetin treatment recognized as adequate for amebic disease. The writer believes that the mere presence of amebae on the surface of the lesion or in the exudate is no more evidence of their participation in the infection than the presence of *Escherichia coli, Clostridium welchii, B. proteus*, or any of the other intestinal organisms is evidence of their activity in the tissues about a fecal fistula. In most of the cases in which amebae have been observed, there has been no accurate determination of the type of ameba, but in those cases having a direct connection with amebic lesion of the liver or gut it is frequently assumed that the organism is *Endameba histolytica*. In the case of Heimburger[75] and in the second case reported by Engman, Jr. and Henry Meleney,[34] there seems to be little doubt that *Endameba histolytica* was present and played an important role in the infection. It is of particular interest to note that in Engman and Heithaus's case,[66] bacterial cultures yielded no growth aerobically. After such a long exposure to contamination, it is surprising that there were not secondary contaminants capable of growth even if the organisms responsible for the lesion had disappeared. The spectacular response both generally and locally to the emetin treatment in this case as well as in Heimburger's case is strong evidence in favor of the importance of the amebae in these infections. In the second case of Engman, Jr. and Henry Meleney, the invasion by the amebae of the whole thickness of the abdominal wall including the muscle is a strong

argument in favor of the activity of the amebae in that case, although there was little response to emetin. The fatal outcome may have been due either to the extent of the amebic lesion in the liver or the virulence of the associated hemolytic streptococcus and *Staphylococcus aureus* in a patient with diabetes. In the first case presented by these authors, although the organism found was almost certainly *Endameba histolytica*, the evidence is not so clear that the ameba played an important role. No cultural studies were made of this lesion so that the factor of bacterial synergism could not be weighed or measured. The amebae were found in the pus and on the surface of the wound but not in the depths of the tissue, nor were they found in the apparently healing ulcer in the gut. It may have been a surface contamination. On the other hand, the temporarily favorable response to emetin may indicate that the ameba played a partial role in the production of the lesion In the case reported by Cole and Heideman,[83] there is still less evidence that the ameba, which was found in the pus but was not classified, was a factor in the infection. The organism was present in the exudate but not in the tissues. There was little if any response to emetin treatment even though the pathology seemed to be localized in the skin lesion The disease was finally controlled only by a wide cautery excision such as is required in the bacterial synergistic gangrene No anaerobic cultural studies were made in this case but the onset and the clinical course were typical of the bacterial synergistic gangrene and similar to the five cases of that type mentioned previously in the lesions of which no amebae were found after careful search by three parasitologists

In all cases of amebic abscess of the liver, the possibility of skin necrosis after drainage must be kept in mind and precautions taken to avoid it. An attempt should be made to protect the skin wound at the time of operation. A two-stage procedure might increase wound resistance to the infection. If a one-stage operation is performed, there should be a complete relaxation of the wound with no attempt to close the skin and subcutaneous tissues by suture. If pathogenic amebae have been found, emetin hydrochloride should be given intravenously. If this medication fails and the disease is localized to the surface lesion, it should be widely excised. In all of these cases, inasmuch as the bacterial symbionts always play a role of greater or lesser importance, a careful bacteriological study of the lesion should be made before operation and cultures taken from various portions of the specimen after excision, should that procedure be necessary.

A case of amebic gangrene of the buttocks, perineum, and scrotum was reported by the author and his brother after careful study from bacteriologic and pathologic standpoints. A thorough search was made for the microaerophilic, nonhemolytic streptococcus, which is the essen-



Fig. 13. Amebic infection. Gangrene of the scrotum and perineum, in which amebae were associated with intestinal bacteria, particularly *E. coli* and nonhemolytic streptococci.

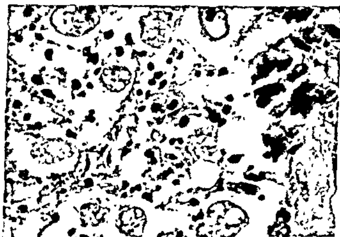


Fig. 14. Photomicrograph showing amebae at the advancing margin of the ulcer. *Note the sharp line of demarcation between the edematous normal tissue infiltrated* with wandering cells (below) and the disintegrated tissue (above). Hematoxylin and eosin stain, × 230 (From the *Archives of Surgery*, Vol 30, pp 980-90, June 1935)

tial organism in progressive, postoperative, synergistic gangrene with which this disease might be confused. This organism was not found, and the pathologic study indicated that the gangrene was due primarily to the lytic action of *Endameba histolytica,* which was clearly demonstrated.

The clinical appearance of this lesion on admission to the hospital is shown in Figure 13 and the pathological section showing the amebae is presented in Figure 14.

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CHAPTER XV

Bacterial Antagonism

WHEN LEEUWENHOEK took some scrapings from his own teeth and examined them in a droplet of water under his microscope, he saw many different forms of animalculi in a conglomerate mixture.[1]

The early bacteriologists were aware of the fact that different bacterial groups frequently occurred together, and they faced the problem of separating the various species from one another. They knew that infectious diseases frequently had characteristic features, which suggested that just one species with some unique function was chiefly responsible. Koch[2] showed the way to separate the individual organisms in a mixture by providing solid media upon which the individuals could form a colony. Koch's postulates have been the criteria for the proof of the bacterial etiology of specific bacterial diseases. It is very difficult to apply Koch's postulates to synergistic or symbiotic bacterial infections. Pasteur was greatly interested in bacterial mixtures and noted that at times the combined existence was favorable (such as anaerobes growing freely with aerobes) and at times unfavorable (such as certain cocci growing with the bacillus of anthrax).[3] Even when grown on solid media it was often found difficult to separate some mixtures of organisms. Many of the early descriptions of the cultural characteristics were made with what we know now were impure cultures.[4, 5] This was particularly true of the anaerobic bacteria, as late as World War I. More recently still, it was found that pure cultures by dissociation would usually produce two or more different kinds of colonies having different cultural, physical, and physiological characteristics, one toxic and the other nontoxic, one pathogenic and the other nonpathogenic, and so on.[6] Then ways and means were found to favor or prevent this dissociation for one purpose or another. This will be discussed more fully in Chapter XXII.

We may be sure that prior to the demonstration that bacteria cause infections and before any efforts were made to keep them away from one another, most infections were due to bacterial mixtures. This was particularly true of surgical infections such as accidental wounds, war wounds, operative wounds, surface ulcerations, hospital gangrene, and so forth.

When bacterial mixtures were first noted, the observers were naturally more impressed with the positive fact of their living together with mutual benefit (symbiosis) than by the negative fact of the absence of some expected strain or the suppression[7] of one by the other (antibiosis). (The term symbiosis was first suggested by de Bary in 1879 and antibiosis by Ward in 1899.[8])And yet there were always certain individuals who kept in mind the possibility of opposing or suppressing pathogenic bacteria by favoring the growth and activity of some antagonistic harmless species. de Bary seems to have been the first to stress the importance of bacterial antagonism.[7]

Among the early workers in this field was Garré[9] who in 1887 used culture plates to show antagonism between *Bacillus fluorescens putidus* and several other species. He called the phenomenon 'metabiotic.' Bouchard[10] in 1889, Charrin and Guignard[11] in 1889, and Blagovestchensky[12] in 1890 found that *Ps pyocyanea* would produce a substance in the nature of an enzyme that could destroy or digest *B. anthracis* and other organisms.

A decade later, Emmerich and Löw[13] confirmed this finding both for pyocyaneus and for some other organisms. They tried to make practical application of it. They named the ferment of pyocyaneus 'pyocyanase,' separated it from the organism, extracted it, concentrated it, and demonstrated that the active principle in a more or less pure state had the power of destroying a number of different bacterial species including cholera, typhoid, diphtheria, plague, anthrax, and the pyogenic cocci. Their first attempts to use pyocanase prophylactically or therapeutically in animals failed. They explained this failure on the theory that the agent was quickly destroyed by the body. When they mixed the pyocanase with blood and other animal tissues, combining it as they thought with certain tissue proteids, they were able to protect their animals repeatedly against fatal infection with anthrax.

The work of Emmerich and Low was confirmed by several other workers,[14, 15] but it did not have wide application because of the variability and instability of the active principle. A few years later, Roger[16] and still later Rettger[17] reported on their studies with *B. prodigiosus.* Rettger found that a dried preparation of this red, pigment-producing organism had marked proteolytic properties when injected into animals but would in small doses protect them against fatal doses of anthrax. However, the toxic properties of the antibiotic agent could not be overcome. Rettger then turned his attention to the bacterial antagonisms of the alimentary canal, and showed that *B. acidophilus* would replace proteolytic organisms there.[18]

It is not surprising that bacteriologists who became aware of bacterial antagonisms should have in mind the possibility of using this property

therapeutically. The problem lay in first demonstrating antagonistic agents against the various pathogenic organisms and then either increasing the potency of the natural antibiotic by extraction and concentration or analyzing it chemically with the prospect of later synthesis.

I. SITES OF BACTERIAL CONGREGATION

To go about trying to find an antibiotic for any given organism requires both knowledge and imagination. First, one must know where different bacterial species congregate and then be able to demonstrate how they behave toward one another. The leading places where bacterial species are likely to come together are:

A. Bacteriology laboratories
B. The intestinal tract of man and animals
C. Sewage
D. Highly cultivated soil
E. Civilian accidental wounds and war wounds

A. ANTAGONISM OF BACTERIA FOUND IN BACTERIOLOGY LABORATORIES

Laboratories equipped for the systematic teaching of bacteriology generally have a file of representative members of each bacterial species that they intend to demonstrate to students. It would take time but not be particularly difficult to combine systematically any two species in the file and to note their behavior toward one another or to grow organisms in suitable media and test filtrates from each one against all the others. If there should be any indication of antibiotic activity by this crude screening, further studies would then have to be carried out with more refined methods. As far as the author knows, such a systematic study has never been done although certain organisms showing antibiotic activity have been widely tested with other species. With the renewed interest in this field, it is probable that such systematic studies will be carried out in several of the well-established laboratories.

The laboratory worker should be constantly on the alert to study evidences of antibiotic activity on the blood-plate cultures of mixed infections. If material from such infections is spread thinly over a blood-agar plate, evidences of either bacterial synergism or antagonism may be observed. If plates containing cultures of pathogenic organisms are left exposed to contamination from the air, these contaminants may either be inhibited by or inhibit the growth of the pathogen. This was done accidentally by Sir Alexander Fleming when he discovered penicillin in 1929.[19] Such accidental success could hardly be expected to come more than once in a lifetime. Sir Alexander recently said at a meeting in



FIG. 1. FLEMING Dr. Alexander Fleming, the discoverer of penicillin, making observations in his laboratory.

New York that it was really an extraordinary concatenation of circumstances. In the first place, he had been interested in bacterial antagonisms and was more or less on the lookout for them. Secondly, he happened to have a culture of staphylococcus on an agar plate That was likely to happen any day in the laboratory. Thirdly, he happened to be a person who was not neat and so left the plate lying on the desk instead of cleaning it up Fourthly, it just happened that a mold fell on the plate



FIG. 2. The colony of *Penicillium notatum* had accidentally fallen on a blood-agar plate on which colonies of *Staphylococcus aureus* had previously grown. Under the influence of the penicillin diffusing out from the penicillium colony the staphylococcus colonies are undergoing disintegration. Photograph of Fleming's original plate. (Courtesy of Sir Alexander Fleming.)

that had the power of producing a diffusable substance that spread out from the mold colony and caused a lysis of the staphylococcus colonies already there (Fig. 2). This was an unusual coincidence of several factors, but as Pasteur once said, 'In the field of observation chance only favors the mind that is prepared.'[20] Sir Alexander Fleming's mind was prepared, his observation was keen, and he had the vision to see at once the possible consequences of this phenomenon. Dr. Helen Jern, working with staphylococcus bacteriophage in the Bacteriological Research Laboratory of the Surgical Department of the College of Physicians and Surgeons, two years later than Fleming,* made the same observation of the antibiotic effect of a penicillium on her staphylococcal colonies. She went at once to the library and found that Fleming had made the discovery previously.

Since the practical value of penicillin has been demonstrated, hundreds of other laboratory workers have exposed culture plates to the air with the hope that some organism might fall upon their cultures that would demonstrate some antibacterial effect. Many active molds have been found, but a much more fruitful search would appear to be a systematic hunt among all available bacterial species for one having potency against the pathogen under consideration.

B. ANTAGONISM OF BACTERIA IN THE ALIMENTARY CANAL

The mouth, as we have said before, is the incubator for many kinds of living organisms, in which the environment is found especially favorable for growth, although there are many organisms that find these conditions unsuitable. Certain organisms like the nonhemolytic streptococci are always found there, whereas others, such as *E. coli* and *C. welchii*, common enough in the intestines, are transient and found only in the mouth occasionally. Apparently some condition in the mouth is antagonistic to their growth. The mouth organisms are constantly being swallowed, and although most of them are killed in the stomach, a few go on into the intestine. Here they increase in numbers down to the ileocecal valve and then decrease again. This increase and decrease in the numbers and kinds of bacteria from the mouth to the anus represent first favorable, then unfavorable, then favorable again, and finally unfavorable conditions, which are probably due in some degree to the changes brought about by the secretions of the glands both in the mucous membrane of the lining of the tract and in the associated secreting organs —the salivary glands, the liver, and the pancreas—all of which glands change the physical and the chemical or enzymatic state of the medium and thus vary the factors that favor the growth or suppression of the contained organisms. The alimentary tract of the newborn infant is sterile, but it quickly becomes contaminated with bacteria. These organisms soon set up an equilibrium or a symbiosis with the infant, which may be called normal. This symbiosis is certainly favorable for the bacteria, which multiply prodigiously, and apparently it is not injurious to the infant. As the child grows to adulthood the flora gradually undergo certain normal changes that have been recognized for a long time, and it is known that they can be modified by changes in diet particularly high or low in protein. Aside from the favorable or unfavorable influence that the host has over the bacterial flora of the alimentary tract, the various bacterial species themselves may have either a favorable or an unfavorable influence on each other. They may be synergistic or antagonistic or they may be indifferent to one another. If the scientist is interested in having his researches in this field directed toward benefit for mankind, he will be more concerned with bacterial antagonisms than with synergisms In order to study these antagonisms, investigators have obtained the various species in pure culture and by combining them under controlled conditions they have studied their interaction.

Probably the most important results of the study of intestinal antagonisms have been the recovery of bacteriophages that cause the lysis of susceptible bacteria. This phenomenon is apparently caused by the action of a living filtrable virus that propagates itself at the expense of the

susceptible organism. The behavior of the virus is essentially different from that of an antibiotic, and will be discussed more fully in Chapter XIX. Upton [21] studied the inhibiting action of *B. bifidus* for *E. coli* and *M. ovalus* in infant stools and came to the conclusion that the inhibiting agents were volatile acids.

Goldmann,[22] and Gundel and Mayer [23] found that the colon bacillus was antagonistic to anthrax. In 1925, Gratia [24] reported that a filtrate from one strain of *E. coli* was a very powerful inhibitor of growth of another strain of the same species, but he did not try its inhibiting action extensively throughout other strains. Later this coli strain lost the power of lysis and then spontaneously recovered it again, indicating the importance of colony variants in the production of antagonistic substances. The lytic agent was not capable of propagation and in other respects differed from bacteriophage.

In 1923 Hall and Peterson found that Botulinus toxin formation could be prevented by filtrates of several of the intestinal organisms containing some product acting independently of the increase of the hydrogen ion concentration.[25] A number of other investigators have singled out two or three of the intestinal strains and have noted their interaction, but the author does not know of any systematic study that has been made to cover all of the possibilities in the alimentary canal.

C. ANTAGONISM OF BACTERIA FOUND IN SEWAGE

In countries where human excreta are used for fertilizing the fields, studies have been made of the flora of septic tanks and night-soil receptacles. It is important from a public-health point of view to know how long pathogenic bacteria and intestinal protozoa can survive under these natural conditions and how they may be destroyed if they survive. It has been found that some die off very rapidly and others live for a very long time.

The Chinese farmers long ago found out empirically that a certain period of waiting rendered this fertilizer relatively innocuous, but in recent years efforts have been made to make it completely safe from the point of view of spreading disease.

In 1900 Houston [26] studied the fate of the bacteria in sewage by spreading it extensively over an area of land and concluded that 'the addition of sewage to soil greatly alters its bacterial composition in respect to *E. coli* and allied forms. But this alteration becomes less and less as time goes on. . . The more hardy soil bacteria seem to oust the more delicate sewage microbes in the struggle for existence.' Savage confirmed this finding by taking cultures from the depths of made soils containing sewage.[27] In 1906 Russell and Fuller [28] reported the rapid disappearance of the typhoid bacillus in sewage or when grown with

sewage organisms, but he did not determine which organisms were responsible for the antagonism. Sewage has been a fertile source of all kinds of bacteriophages, but it has not been as thoroughly studied for antibiotic substances as it deserves.

D. ANTAGONISM OF BACTERIA FOUND IN THE SOIL

When man and other animals deposit their excreta on the land or when they die and are buried in the ground and their bodies undergo disintegration, the intestinal bacteria have an opportunity of being spread abroad very widely through the soil by the movement of water. Many of them die because of lack of food supply, many are killed by organisms in the soil (discussed more fully below), but many are taken up into the digestive tract by insects or other animals and find again a favorable environment for multiplication. If they are carried away by rain water, they may reach brooks, streams, lakes, or rivers and eventually reach the water supply of towns or cities. Then they are either destroyed by purification systems or enter again the alimentary tracts of man or animals by way of water or food. Thus the cycle is completed.

Many investigators have studied various phases of this cycle, particularly the agricultural and public-health bacteriologists. Anthrax, the devastating disease of sheep, and typhoid fever, the dreaded disease of man, very early came under these investigations. Frankland in 1893 [29] showed that B. *anthracis* would live for 20 to 51 days in deep well water or in sterilized polluted water, but would die off in 9 to 13 days in unsterile surface water. Jordan in 1940 [30] found that *Eberthella typhosa* would live 15 to 25 days in sterilized tap water, but only 4 to 7 days in unsterilized tap water and only 1 to 4 days in raw canal or river water. These authors concluded that other living organisms in the polluted water were antagonistic to the pathogens and destroyed them or starved them to death.

The destruction of sewage bacteria by resident soil organisms suggested the possibility of finding some active principle produced by one or more of the soil bacteria that might be utilized to prevent the growth or activity of some of the human pathogenic microbes. Following the lead of earlier workers (de Freudenreich particularly,[31] Olitzky,[32] Laws and Andrews,[33] and Martin [34]) Frost [35] in 1904 reported on his elaborate studies with the typhoid bacillus (Fig 3). He selected the saprophytes most likely to meet with the typhoid bacillus in nature and used many different ingenious methods to show their antagonism. His principle method was to grow the typhoid bacillus in a collodion sac (containing water or broth) suspended in a broth medium in which a sample of soil or polluted water was inoculated. He varied the time of inoculation of the typhoid bacillus and the soil, but in every instance the typhoid bacillus was either pre-



FIG 3. FROST. Dr W. D Frost made many careful studies which demonstrated the ability of soil organisms to destroy the typhoid bacilli from human excretions (Courtesy of New York Academy of Medicine)

vented from growing or killed if growth had already been established Frost then went on to identify the organisms in the soil and in the polluted water and to test them out individually against the typhoid bacillus on agar plates. Some of these were found to be antagonistic, particularly strains of the pyocyaneus and proteus groups. He found that the antagonistic action was best at body temperature, that dextrose was advantageous in the media, chiefly because it stimulated growth, that the presence or absence of oxygen was immaterial, and that it developed whether the reaction was neutral or slightly acid or alkaline. He discussed the possibilities of the nature of the antagonism, considering the exhaustion of food supply, enzyme action, specific poisons, acid or alkali production, and came to the conclusion that it was something other than all these.

Since Frost's careful studies, there have been many other reports—now so numerous that they may well be grouped together into four main categories: (1) Gram-negative aerobic nonspore-forming rods of the Pseudomonas (pyocyaneus) group, lipoidal in character;[36] (2) spore-forming rods belonging to the *Bacillus mycoides* or *mesentericus* groups, capable of lysing certain Gram-positive bacteria but limited in range[37, 38] and

and later isolated from this soil an aerobic Gram-negative sporulating bacillus that he called *Bacillus brevis* and that produced a soluble principle extremely toxic for Gram-positive bacteria. He called this soluble principle tyrothricin, and found that it contained two different active principles, to which he gave the names gramicidin and tyrocidin. Tyrothricin has been successfully used to combat a persistent streptococcal mastitis of cattle. Gramicidin has also been used with some success in localized human infections, but on account of its toxicity is restricted to local application in limited quantity. About the same time Hoogerheide [48] isolated from another soil bacillus a similar active principle, but it too was toxic and its use was limited to local application in local infections—with some reported successes.

In 1940 Waksman [49] reported on certain bacteriostatic and bactericidal substances produced by soil actinomyces (Fig. 4). By the use of agar suspensions of the test organisms poured into Petri dishes containing various dilutions of soil suspension, he was able, after incubation, to demonstrate inhibition by clear zones and then to isolate the organisms causing the inhibition. He demonstrated the antibiotic effect on *E. coli, Brucella abortus*, and, later on, many other species.[50] The active principle from the actinomycetes, he called actinomycin, later streptomycin. Commercial production of this antibiotic has been difficult, but it is now available for clinical use and has demonstrated its usefulness (see Ch. XXII).

E. ANTAGONISM OF BACTERIA FOUND IN CIVILIAN ACCIDENTAL WOUNDS

During the recent study of the prevention of infection in civilian accidental wounds, to which reference was made in Chapter VII, the author's unit at the Presbyterian Hospital, New York, made a search for antibiotic activity among the bacteria found in the débrided tissue from many of the wounds. Such agents demonstrated their presence by showing growth of several different species on the direct immediate plates with an inhibition of one or more of these species when grown in the primary broth cultures. Several of these were found by Miss Balbina A. Johnson, and one proved to be highly potent, wide in its bacterial range, water-soluble, thermostable, and nontoxic. This species was derived from the compound-fracture wound of a patient named Tracey. It was produced by a large Gram-positive aerobic spore-forming rod of the *B. subtilis* group and has therefore been called Bacitracin. The preliminary observations of its local application to clinical infections resemble the effect obtained with penicillin. The first hundred cases of surgical infections treated locally with Bacitracin have been recently reported by the author. The results were favorable in 88 per cent of the cases.[51] The organism producing this antibiotic sporulates freely on solid media. The spores are

oval and either eccentric or subterminal. Milk is slowly digested, gelatin is liquefied. Indol is not produced. Nitrates are reduced, and it contains catalase. It grows readily as a thick pellicle on the surface of fluid media and liberates the antibiotic substance into the media. Filtrates are produced equally well from cultures grown in Bacto tryptose broth, Savita broth, and amigen. A synthetic glutamic acid medium has been developed in which the active substance is produced. This simplifies the process of extraction and analysis. The antibiotic agent passes through Berkefeld, Chamberland, Coors, and Telas filters. The filtrates are alkaline pH 8.4-8.6. There is no loss of activity when the filtrate is adjusted to pH 7.2-7.4. The filtrate reaches its maximum antibiotic activity after 2 to 4 days' culture. The filtrate strongly inhibits the growth of numerous strains of the hemolytic streptococcus, nonhemolytic streptococcus, pneumococcus, gonococcus, meningococcus, *C. welchii*, *C. septicum*, *C. sordellii*, and other Gram-positive organisms but has no effect on Gram-negative rods. It is less strongly inhibiting for the hemolytic *Staphylococcus aureus* and *C. histolyticum*.

The active substance is extracted from the 3-day culture filtrates by means of butanol, which takes it from either acid or alkaline solution. Further concentration is effected by distillation of the organic solvent and by subjecting the aqueous residue to fractional precipitation with ethanol. In this way, a concentration of over 100-fold has been secured. The active material is readily soluble in water and in ethanol; sparingly soluble or insoluble in acetone, ethyl acetate, chloroform, and benzene. The cell-free filtrates are thermostable without loss of activity after being kept at 100° C. for 15 minutes. They show no evidence of toxicity on intraperitoneal or subcutaneous injection in mice or guinea pigs. Twenty mice received 2 cc. daily for 15 days without ill effect, and 2 guinea pigs received, respectively, 10 cc. and 20 cc. for 21 days without any ill effect. Mice have readily tolerated as much as 1 cc. of the 100-fold concentrate on 2 successive days without any sign of toxicity. Mice have been protected against 10,000 M.L.D.s of an intraperitoneal injection of hemolytic streptococcus, strain #6303 M.V. by repeated subcutaneous injections of the undiluted filtrate. Bacitracin is a neutral substance for it can be extracted from aqueous solutions of pH 2 to pH 9 with butanol. It is insoluble in organic solvents, with the exception of the lower alcohols; it is fairly diffusible, it is not destroyed by boiling for 15 minutes, but it deteriorates with time in an aqueous solution. By the standard procedure it can be obtained as a yellowish powder of an average activity of 25 to 35 units per mg. Preliminary tests indicate that if larger quantities become available, it will not be difficult to increase the state of purity. It is stable toward the action of pepsin and commercial trypsin (a mixture of the pancreas enzymes). The chemical properties of Bacitracin seem to

indicate either a polyhydroxy or amphoteric compound of low molecular weight.[52] Its clinical usefulness is described in Chapter XXII.

II. THE NATURE AND MODE OF ACTION OF BIOLOGICAL ANTAGONISM

When two organisms are in juxtaposition, they are in more or less competition with one another for food. If one is able to utilize this food faster than the other, it may cause the other to starve to death or go into a resting stage. In the course of its metabolism, one organism may develop excretory products or poisons that will destroy the associated organism. This may be brought about by changes in surface tension or pressure, or the hydrogen-ion concentration or the oxidation-reduction potential of the environment, or specific enzymes may be produced that interfere with the various metabolic requirements of the victimized cell. These antagonistic substances may be of countless variety and it is often extremely difficult to determine just how they work in any given case. Arnaudi et al.[53] believed that it could all be explained on a physical-chemical basis. Different organisms growing alone bring about certain changes in the media. Arnaudi demonstrated such changes as surface tension, pH and electric conductability and thought that there were probably others. If two organisms are growing together, the changes initiated by one may be inimical to the growth or life of the other. Waksman[54] has recently summed up their action as follows:

> All of these various antagonistic substances have been called antibiotics. They may be produced by viruses, by bacteria, by fungi, or higher plants, and they may act on viruses, bacteria or fungi or on protozoa or higher animals. Some antibiotics can be separated from the producing agent and act independently while others can only act in close association with it or be liberated from it when the organism dies. The production of the antibiotic depends to a large degree on the composition of the medium in which the organism is grown, the phase of its growth and the temperature of incubation. There must be a source of protein and carbohydrate, essential salts and vitamins and the hydrogen-ion concentration and oxidation-reduction potential must be within narrow limits. Optimum quantities of each of these have to be worked out for each individual antibiotic in order to produce the greatest yields. The organisms producing these antibiotic substances tend to dissociate, some progeny being highly active and some quite inert. In preparing these agents this must be constantly kept in mind.

As one would expect, most of the substances that have been shown to be antagonistic to bacterial growth are toxic for man and animals because many of the cellular functions of man are similar to the cellular functions of bacteria. To be injurious to any given species of bacteria and

not to its host, the antibiotic must interfere with one of the essential functions of the former that it does not have in common with the latter. There are very few antibiotics that satisfy this condition and that can therefore be considered for general therapeutic use. Penicillin is the agent of highest potency over many bacterial strains that has so far proved generally safe. Streptomycin and Bacitracin seem to fall in that same category. Further clinical experience with these agents is gradually accumulating. If toxic antibiotics are used locally there must be a wide margin between the toxic and the therapeutic levels within which to operate.

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CHAPTER XVI

The Defense of the Human Body against Bacterial Infection

I. PRIMARY DEFENSES OF THE BODY

A. Surface Impermeability

The external surface of the body, except for the eye, is covered by a horny layer of dead, dried, epithelial cells, varying considerably in thickness as well as in pliability in different parts of the body. These cells form a dense barrier against the penetration of external substances. Through the skin, there are small channels for the exit of the sweat and sebaceous glands. At these points the protection is relatively weak. The inner surfaces of the body that are continuous with the external surface are lined with mucous membrane. The nasopharynx, the sinuses, the mouth, the respiratory tract, the alimentary canal, and the genitourinary tracts are covered by an epithelial coat of very different character from that of the skin. Instead of being hard and dry, it is soft and moist. In places like the mouth, the esophagus, the bladder, ureters, kidney pelves, and vagina, this membrane is, in certain respects, like the skin, the surface layers being rather for protection than for secretion. The horny layers, however, are absent, the living cells are nearer the surface, and, except for the bladder, ureters, and kidney pelves, there is a discharge of glandular secretions upon the surface. Thus these membranes are probably less resistant to the entrance of organisms than is the skin. At the same time they are protected to some extent by the fact that they are internal and are not as subject to trauma as are the external surfaces. In those parts lined by epithelium of a columnar or cuboidal type, the layer of epithelium is still thinner, and the number of openings for the glandular secretion are infinitely more numerous, so that they, in turn, represent a still weaker barrier to the introduction of external organisms. It is generally believed, however, that the intact skin and mucous membrane relatively seldom permit the passage of organisms. If organisms get into the glandular ducts and if these ducts are blocked off, the organisms may grow and produce an infection. This is particularly true of virulent types of organisms, which are specifically adapted, apparently, to the membrane with which they are associated in disease processes, as, for example, the

staphylococcus to the skin, the typhoid bacillus to the intestine, the gonococcus to the genital tract, the diphtheria bacillus to the throat.[1] However, in the great majority of cases and for the great majority of organisms, there must be a slight break in the primary defensive wall or a physical change in its surface before they can pass this barrier.

B. SURFACE SECRETIONS

The bactericidal action of the sweat and sebaceous glands is of relatively little importance. The lacrimal secretion is said to be slightly antiseptic,[2] but the chief means of disposal of organisms deposited on the conjunctivae is the washing effect of tears. The salivary secretions may be antiseptic to some degree,[3] but the secretion of the other glands of the nose, mouth, and esophagus have little or no antiseptic action. However, the mucus secreted by these membranes plays a part in their disposal, for as it collects it gathers up the surface bacteria and they are carried down into the stomach, where most of them are destroyed.

By far the most important secretion for the destruction of bacteria is the gastric juice. This bactericidal action is due largely to the acidity of this fluid, which is beyond the acid death point of the great majority of bacteria.[4, 5] Many observers, notably Cushing and Livingood,[6] have found that bacteria decrease in numbers in the stomach. Miller,[7] introducing bacteria into the stomachs of animals, was able to demonstrate a progressive diminution of organisms to the point of sterility. It has been claimed by some that gastric secretion has some antiseptic action aside from its acidity, but this is of minor importance. If fluids are taken on an empty stomach they may pass over into the duodenum without giving the gastric juice time to destroy the contained bacteria. Of more importance, however, are those conditions of achlorhydria in which the stomach fails to perform the important function of destroying ingested bacteria Then large numbers go over into the intestine and not infrequently cause moderate or severe bowel disturbances. Normally the acid-resistant nonhemolytic streptococcus survives and enters the duodenum, where it is regularly found in small numbers.

For some time it was thought that the bile had an antiseptic action,[8] but there is very little evidence to favor this contention.

The antiseptic action of the digestive juices of the intestine is also of relatively minor importance.[9] However, if the fecal stream is diverted, the bowel will rid itself of the residual organisms in a relatively short time.[10-12]

It must not be forgotten that in the intestine the bacteria are also subject to the inhibitory action of other organisms present. Rettger and [Ch]eplin[13] have shown that the body is defended against the action of [so]me pathogenic organisms by certain innocuous saprophytes of the

intestine; and in the colon this may be the principal cause of the great destruction of bacteria,[14] although the secretion of the membrane may play a part.

In the vagina, the secretion has been demonstrated to have a definite antiseptic action for most of the common organisms except the gonococcus and one or two innocuous organisms such as the bacillus of Doderlein, which latter types may be considered to be normal inhabitants of the vagina.[15, 16] The antiseptic action in the vagina is probably due to some extent to the lactic acid production of Doderlein's bacillus.

C. Mechanical Removal of Bacteria

The frequent desire of human beings to wash themselves gives a large measure of protection against the entrance of bacteria. Certain other functions incidentally remove many organisms from areas where they might do harm if they were retained. This applies particularly to the conjunctivae, where the lachrymal secretions constantly bathe the eyeball; to the bronchi and trachea, where the cilia carry bacteria and other foreign bodies outward; to the intestines, from which enormous numbers of bacteria are mechanically removed; and to the vagina, purged by the menstrual flow and normal secretions.

II. SECONDARY DEFENSES OF THE BODY

A. Local Conditions at the Point of Entrance

As soon as the bacteria have passed the barrier of the surface covering of the body, either the skin or the mucous membrane, they find themselves in direct contact with tissue cells—some of which may have been killed or damaged by the injury that caused the break in continuity, but some of which are living and are fully able to mobilize their defenses against the foreign agents of disease. There may be extravasated blood elements and injured blood vessels, but there are also intact blood vessels and normal blood cells. The less damage done to the tissues at the time of the entrance of the micro-organisms the more adequate the defense of the body against the organisms. Usually, as Zinsser[17] has said, 'the invading cell meets the invaded one under conditions peculiarly adapted to the activities of the latter,' giving the body the advantage in a struggle about to begin. However, if there is considerable damage to the tissues, the defensive mechanisms are handicapped and the bacteria are better able to maintain themselves, colonize, and become established in the tissue.

The path of introduction of any given micro-organism is of considerable importance, because upon it depends, to a great degree, the favorable or

unfavorable environment for its growth. For instance, the pyogenic cocci, if introduced into the skin, have a greater chance of causing infection than if they are swallowed; but the bacilli of typhoid, of cholera, and of dysentery are inhibited and favored in just the opposite way. Organisms entering the tissues through the skin or mucous membranes immediately encounter a protective mechanism that readily destroys most bacteria, although certain of the staphylococci and hemolytic streptococci are able to survive. If these cocci are swallowed, however, they generally have no effect on the body, whereas the enteric organisms usually cause typical infection.[18]

Any bacterium may accidentally enter the tissues of any animal. This is merely a coincidence of two living beings both more or less ubiquitous. In the large majority of cases in which bacteria have passed the first general barrier of the surface defense, no infection of the host results. The explanation of this negative result lies, for the most part, in mystery.[19] We know that there are certain physical conditions that are unfavorable to bacteria, as, for example, certain degrees of temperature and certain levels of hydrogen-ion concentration, but even when these are favorable, there are other forces that we cannot explain which still prevent the growth and multiplication of bacteria. These are summed up in the term 'natural resistance.' Why some animals are never spontaneously invaded with many micro-organisms that cause extensive and fatal ravages in others[20] is a question that the Science of Immunity is trying to answer. Little by little the mechanism of this process is yielding to the persistent researches of many men who are not only seeking to learn the facts of life but also are trying to develop methods for transforming susceptibility into resistance against disease.

It must be remembered that any animal infection is the result of the struggle between two variables, one the pathogenic powers of the bacterium and the other the resisting powers of the host. These variables in turn are each modified by the local conditions in which this struggle takes place. 'Therefore,' as Zinsser said, 'the conceptions "resistance," "immunity," and "susceptibility" are relative terms which can never be properly discussed without consideration of all modifying conditions.'[21]

B. NATURAL IMMUNITY

The term 'natural immunity' is used to cover all the circumstances of the condition that renders a normal animal inherently insusceptible to infection even after bacteria have entered the tissues. Natural immunity may be a characteristic of the species, the race, or the individual. It is a common observation that certain individuals seem to be naturally resistant to infections while others seem particularly susceptible. One would expect such resistance to be nonspecific and apply to all infections.

Webster[22] came to this conclusion when he found, among his controlled mice colonies, parallel responses to such widely different harmful agents as *Bacillus aetrycke* and mercuric chloride. On the other hand, Hill, who studied the genetic factors in resistance to infection, found three strains of mice equal in their resistance to *B. enteritidis*, and unequal in their resistance to other pathogenic organisms.[23]

When one realizes how soon after birth animals and man come into contact with the bacterial world and start reacting to it, as we know they must because of the wealth of our experience from studying artificially controlled contact between bacteria and animals, it is obvious how difficult it is to separate the factors of natural from acquired immunity. From the time of birth the experience of one individual begins to be different from that of every other individual. Even before birth some of the experiences of the mother in her contact with disease are transferred to the offspring through the blood. However, there is a certain amount of experimental evidence to indicate that resistance may be handed down from the male parent through the genetic pattern so that there must be some inherent resistance or susceptibility that may rightly be called 'natural.'

It has been claimed that the greater susceptibility of the Negro race, compared with the white, to tuberculosis is on a genetic basis, but it is fairly well proved that the Negro's resistance to yellow fever is due to a resistance built up by mild childhood disease. The work of Sawyer[24] and his associates in endemic areas of yellow fever amply confirm this point of view, previously held by Hahn.[25] The same explanation is probably valid for the resistance of the Negro to malaria and of the Mexican to smallpox and typhus.

Nevertheless the process of natural selection by which the fittest survive, weeding out by death the more susceptible individual, may result in a genetic pattern of resistance to infection.[26]

True it is, that certain infections, entering a community where they have not previously existed, cause ravages that they cannot produce in endemic areas, as has happened repeatedly with such diseases as syphilis and tuberculosis. A corollary of this is the well-known fact that Eskimos fall easy victims to tuberculosis when they come into temperate climates. But even in generally resistant communities immunity is a relative term. Individuals may be able to resist a small dose of organisms but be overwhelmed by larger doses. Or the normal resistance of an individual may be temporarily lowered by injury or chilling or fatigue, and he may fall victim to an infection that he would otherwise resist.

Early investigators in the field of immunology pondered on these facts, which were generally known.

Pasteur had been able to protect susceptible animals by preliminary



FIG. 1. METCHNIKOFF. Dr. Elie Metchnikoff was the leader of the cellular school of immunologists, and made many observations supporting his theory. (Courtesy of New York Academy of Medicine)

injection with attenuated or dead vaccines, and it was thought that the demonstrable changes in the cells and body fluid in such animals might explain in a similar fashion the natural resistance to infection.[27]

In studying these changes, two schools of thought developed, one led by Metchnikoff,[28] which focused attention on the changes in the cells, particularly the wandering cells of the blood and those cells that accumulate as exudates in areas of inflammation, and the other led by Flügge and Mittall,[29] which stressed the changes demonstrable in the body

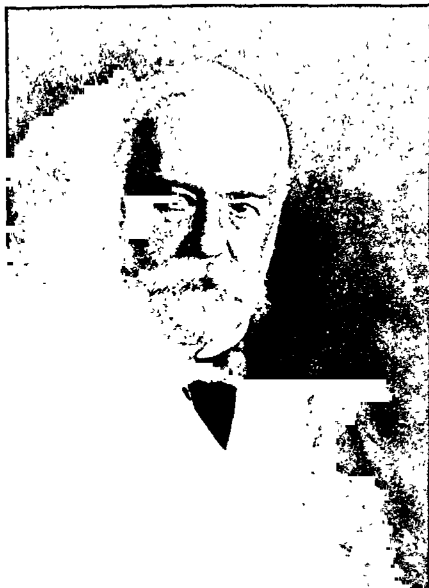


FIG 2. FLÜGGE Dr Carl Flugge was the leader of the humoral school of immunologists, and set up a lively rivalry with Metchnikoff. (Courtesy of New York Academy of Medicine)

fluids, particularly the serum and plasma. This sometimes friendly, but frequently acrimonious, competition brought out a great volume of experimental work, and each leader became supported by a host of disciples. Evidence piled up first on one side and then the other, but neither could claim perfect consistency. Finally, it became necessary to admit that neither was wholly right, but the two points of view could



FIG. 3. LADY MONTAGU. Lady Mary Wortley Montagu, after seeing the devastation of smallpox in India helped to popularize vaccination in England. (Courtesy of New York Academy of Medicine)

smallpox exudate from man (Fig. 4).[37] It was almost a hundred years after Jenner, however, that Pasteur observed the attenuation of virulence of the chicken cholera organism and demonstrated the protection afforded by inoculation with the attenuated strain.[38] 'This was the real beginning of the science of immunity.'[39]



FIG 4 JENNER. Dr. Edward Jenner firmly established the value of vaccination against smallpox (Courtesy of New York Academy of Medicine)

Acquired immunity, unlike natural immunity, may sometimes be passively transferred from one animal to another, but this applies only in conditions of immunity to those diseases in which protective substances are present in the serum of the immune individual The concentration of these substances in the serum subsequent to the recovery from the infection, or following a period of voluntary active immunization, varies considerably with different diseases and different individuals. The serum

remains potent for a much longer time following recovery from disease than following artificial immunization. In the former it may last for years, in the latter it may not persist for more than a few weeks or months.

Long after demonstrable antibodies have disappeared from the serum, the individual with acquired immunity is resistant to the disease. This would seem to indicate that there is some fundamental alteration of the cells originally responsible for the production of the antibodies that renders them potentially available for further rapid mobilization of fresh antibodies should the antigen again present itself.

The list of diseases against which man derives a lasting immunity following one attack are strangely enough all so-called medical diseases. Infectious diseases caused by the pyogenic or necrotizing organisms, which render them amenable to surgery, because of their tendency to localize, for the most part fail to arouse in the patient any antibodies of a lasting character; none, at least, that can be demonstrated in the blood serum by any tests we now know or that give any indication of their presence by protection against subsequent attacks.

It has been possible recently to show a certain amount of antitoxin in patients suffering from staphylococcus infections.[40] They usually have higher titers than normal individuals so that we cannot be sure that their presence indicates any real defense against the infection. Similarly, the demonstration of antistreptolysin in human serum is an evidence of infection rather than of immunity.[41] This would seem to indicate that recovery from these diseases is due to a local rather than to a general defense against the organism and discourages any hope that one might have of building up any immunity in human beings by the inoculation of these organisms in the form of vaccines or any immunity in animals that might subsequently be transferred by blood or serum to human beings infected with the disease.

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The Mechanism of the Secondary Defenses of the Body

THE MECHANISM of defense against organisms by natural immunity is not well understood. Extensive studies have been made in this field, but many of the factors that undoubtedly play a part in this resistance to infection are not known.[1] We know that bacteria virulent for one race or one species may be entirely impotent when injected into the tissues of others even in large quantities. Hopkins and Parker[2] showed that a naturally resistant animal like the cat will rapidly destroy hemolytic streptococci in the lungs and liver when the organisms are injected intravenously. This demonstrated that these organs can quickly clear the blood stream of susceptible bacteria, but it did not explain the inability of organisms to gain a foothold at the portal of entry into naturally immune animals. Here the defensive role may be played by the fixed tissue cells, the wandering cells, or the humoral elements in the blood. No way has been found to measure this defense except the rather uncertain measure of the phagocytic power of the leukocytes, which is aided in some manner by the opsonic power of the serum. D'Herelle believes that phagocytosis explains the whole mechanism of natural immunity,[3] but it is not as simple as that, because no consistent parallelism exists between phagocytic power and natural immunity.

The problem is one that does not easily lend itself to conclusive experimentation, because the conditions cannot be entirely controlled. Unless the bacterial environment of the experimental animal can be thoroughly controlled from birth to the time of the experiment, it is impossible to tell whether a resistance to infection that may be demonstrated is natural or acquired. Such experiments have been carried out along the lines of heredity by Webster,[4] Hill,[5] Greenwood et al.[6] and others who have demonstrated the possibility of breeding races of animals in such a way as to render them either resistant or susceptible to infection. Webster concluded that the resistance was general to all types of infection, but Hill showed that a species might be resistant to one type of infection and susceptible to others. Neither worker was able to determine unqualifiedly the mechanism of the resistance, although it seems to be on a genetic basis.

In acquired immunity, the mechanism seems to be very much more complex, but because we can control the experimental conditions, it is much easier to study its different phases. D'Herelle[7] says: 'The experimental disturbance in a refractory animal and the natural or experimental disease in a susceptible animal, caused by the same bacterium, are fundamentally different.' In each, there is a disturbance of equilibrium, but in the latter there is an affinity of the products secreted by the bacterium for certain cells of the body that results in a cellular disorganization, whereas in the former, there is no affinity whatsoever for the body cells.

With regard to acquired immunity, there has been the greatest possible difference of opinion, as represented by the Metchnikoff[8] and Flügge schools,[9] the former maintaining that the body cells were the chief factors in the defense against the bacteria, and the latter contending that the body fluids were the chief elements. Each of these schools exaggerated its own and minimized the other's findings in support of its individual theories; and in the middle eighties almost all of the workers in Europe took sides on this very important question. Discussion was fruitful in bringing out many ingenious experiments to prove this or that theory; but it was difficult to get an unbiased view of the question. It was obvious, however, that certain facts favored the humoral theory of immunity, and also that certain other facts favored the cellular theory of immunity. These facts could be finally reconciled only by recognizing that neither cells nor fluids play the only role in immunity; that there is between them an interdependence of the utmost importance. It may be mentioned in passing that the earlier theories of immunity, namely, those of Pasteur and Nencki, did not have experimental backing. Pasteur[10] thought that, with the first attack, bacteria used up certain elements in the body necessary to their growth. This was known as the 'exhaustion theory.' Nencki[11] suggested that just as bacteria are inhibited by the production of their own waste products in the test tube, so their growth might be overcome in the body by an accumulation of their metabolic products. This was called the 'retention theory.'

We need not go into all of the very enlightening experiments carried out and presented in support of either the cellular or humoral theories. It should be noted, however, that these experiments have been productive, incidentally, of the most illuminating facts. The following points stand out with striking interest.

Von Behring[12] was the first to show that for diphtheria and tetanus, at least, the immune properties of the cell-free blood serum were the all-important factors of immunity against organisms producing true exotoxins, and specificity was recognized for the first time by him in 1890, the specific antibodies being called antitoxins. Later it was observed that



FIG 1 VON BEHRING Dr. Emil Adolph Von Behring demonstrated the value of passive immunity produced by the injection of toxins into animals and the treatment of infectious diseases with antitoxins (Courtesy of New York Academy of Medicine)

the serum of rabbit's blood rapidly destroyed anthrax organisms, although the rabbit is very susceptible to the disease, while the serum of dogs had no effect on anthrax organisms, although the dog is highly resistant.[13] These and other facts marshaled by many observers have now led us who are away from the immediate prejudices of the old argument to appreciate the fact that in the reactions of immunity both the body fluids and the cells play important roles.

I. THE ROLE OF THE WANDERING CELLS IN IMMUNITY

In the lowest animals, the single cell is called upon to perform all necessary functions. As the scale of life rises and bodies become more complex, the cells take on a division of labor. Many of them become differentiated to perform a special function, and thereby lose some of their primitive characteristics. Within the body, however, are cells that behave, in many respects, like the unicellular organisms, and these wander about the body. Their derivation is of considerable interest (Fig. 2). They are carried about by the circulation of fluids, but they pass in and out of the walls of capillaries in response to certain chemical and physical changes in their surroundings. At times, they take over the function of certain fixed tissue cells and may later revert to their wandering. They differ from the differentiated tissue or organ cells of the body by having the power to take up particulate matter. There are two groups of these cells: first, the polymorphonuclear leukocytes, called microphages, concerned primarily with the ingestion of bacteria, especially in the acute stages of inflammation; second, the large mononuclear leukocytes, clasmatocytes, and endothelial cells, as well as certain fixed tissue cells, chiefly concerned with the removal of cellular debris, foreign bodies, and the bacteria of low-grade infections. These are called macrophages. Certain other cells that remain as fixed tissue but that phagocyte bacteria will be considered later.

The polymorphonuclear leukocytes are not present to any degree in normal tissue. They are present in the blood, and in normal individuals their number remains fairly constant, fluctuating to some extent from hour to hour throughout the day, but normally remaining within certain limits, which may be given roughly as 4000 to 7000 per cubic millimeter of blood. Their behavior in certain circumstances of inflammation has been observed many times both in experimental and pathological material. Their reaction can be seen in the microscopic examination of a frog's mesentery. With the introduction of the excitant, the blood current at first becomes rapid, the blood vessels dilate, and then the current becomes slow; leukocytes are seen to cling to the capillary walls, and then by ameboid movements to pass through the walls. They then approach the foreign substances and take them up or flow around them. (See Figs. 3 and 4, pp. 506 and 507.) Various stages of the process can also be seen in tissue taken from areas of acute inflammation (see Fig. 25, p. 429).

What governs the movement of leukocytes toward a focus of inflammation is not yet fully known. The degree to which they react to the irritant and take up the bacteria varies considerably with the nature of the bacteria. Some bacteria are taken up in great numbers with great rapidity

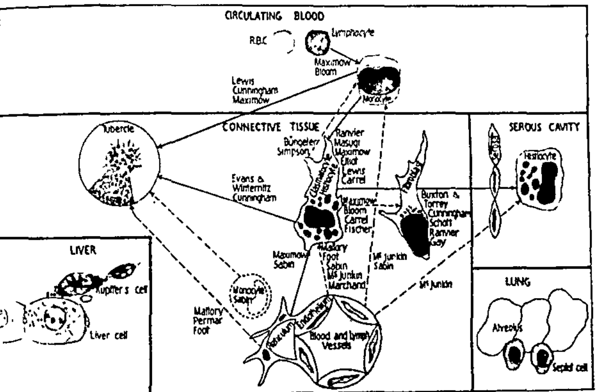


FIG. 2. Indicating the lines of intergenetic relationship between type cells of the 'macrophage' or 'reticulo-endothelial' system of mammals. The heavy lines indicate the most widely accepted lines of transformation of one type cell to another, whereas dotted lines show less widely accepted or more recently proposed transformations. The principal authorities for these relationships are given (Courtesy of Dr. F. P. Gay)

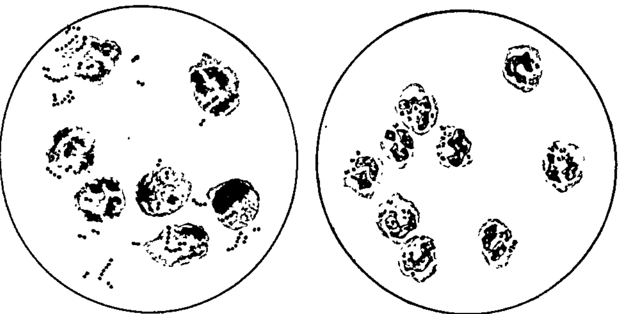


Fig. 3. (a) Positive chemotaxis and ingestion of a virulent streptococcus by microphages which disintegrate under action of leucocidin formed in the phagocyte. (b) Positive chemotaxis and ingestion of meningococci by microphages of human cerebrospinal fluid. The ingested micro-organisms resist digestion and the microphages are relatively unaffected. (From Dr. F. P. Gay, *Agents of Disease and Host Resistance*, Thomas, Springfield, Illinois, 1935)

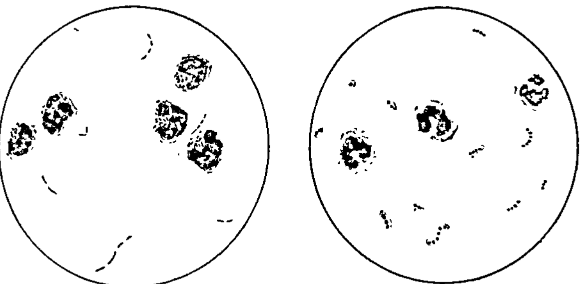


FIG. 4. (a) Positive chemotaxis; Ingestion, and Digestion of *V. cholerae* by rabbit microphages which remain relatively intact. (b) Negative chemotaxis exerted by virulent, capsulated Type III pneumococcus on polymorphonuclear cells of the rabbit. (From Dr. F. P. Gay, *Agents of Disease and Host Resistance*, Thomas, Springfield, Illinois, 1935)

and become disintegrated within the phagocyte; others are taken up by the phagocyte but continue to grow within it and eventually destroy it, becoming free in the surrounding tissue again. With certain infections, as for example with the virulent gas-forming anaerobic organisms, there seems to be an actual repulsion of the polymorphonuclear leukocytes. In some cases, the bacterial cell will attract the phagocytes, whereas some of its products repel them. This is true of tetanus spores and tetanus toxin. The attraction or repulsion of leukocytes often differentiates virulent from avirulent bacteria of the same morphological type or the same species.

A. CHEMOTAXIS

The movement of leukocytes is a very interesting phenomenon and has been given a great deal of study. The attraction of leukocytes is called *positive chemotaxis*, and the repelling of leukocytes, negative chemotaxis. The power of stimulating positive chemotaxis is a property not only of bacteria but also of bacterial products, of disintegrated tissue cells, including leukocytes and red blood cells, and even chemically inert substances, such as carbon particles. Negative chemotaxis is apparently brought about by certain of the products of virulent bacteria and the organisms causing some of the chronic diseases. The etymology of the word indicates a chemical action, and for a long time some thought, and many still believe, that certain chemical substances diffuse from the source of infection and enter the circulation, and thus are supposed to call forth the leukocytes from the bone marrow and the other places of their manufacture. It has been demonstrated, however, that such a phenomenon may be explained largely on a physical basis, and many observers now feel satisfied that surface tension plays the greatest rôle in 'chemotaxis.' Zinsser and Wells both favored this latter opinion. Zinsser[14] explained it in some such manner as this: Particles are held together by cohesion; solids, liquids, and gases vary markedly in this respect, heat tends to increase the motion of particles and to decrease the cohesive forces; cold, on the other hand, increases the cohesion. Large bodies of fluid take the shape of the containing vessel; small particles tend to take a spherical form. The cohesive force is directed inward toward the center. Thus the surface is reduced to a minimum and the tension tends to be the same on all parts of the surface. However, the surface tension is modified to a certain extent by the surrounding substances. Anything tending to lower the surface tension at any point on the surface causes a movement of the particle in that direction until an equilibrium is re-established and the tension on all sides again becomes equal. The surface of the cell that is not fixed by processes that bind it to other cells is subject to this same law of surface tension. Anything lowering the surface tension on one side will cause a movement of the cell in that

direction. Cellular movements may be simulated by a very ingenious experiment. If chloroform is added to water and a piece of glass covered with shellac dropped into the mixture, the chloroform will surround the particle, dissolve the shellac, and extrude the glass.[15] Most diffusible substances will lower surface tension on the side on which they are acting. With negative chemotaxis, on this basis, it must be assumed that the surface tension is increased in some way on the side of the substance causing the repellent action. The chemical substances produced by bacteria and by the disintegration of cells diffuse in all directions. When they meet the capillary wall, they pass through. The leukocytes passing by, move toward the capillary walls because of the lessened surface tension on this side (see Chap. XIII, Fig. 25). They pass through and keep moving, for the same reason, to the site of the production of these chemical substances.[16] Leukocytes with the most cytoplasm are most active in migration, and Wells gives this as the reason why polymorphonuclear leukocytes are affected more than lymphocytes. General leukocytosis may be explained in the same way. Chemotaxic substances from the area of inflammation enter the blood stream and in a very diluted form pass through the bone marrow, thus attracting leukocytes into the blood stream, where they circulate until they reach the point of manufacture of the chemotaxic substances.[17] D'Herelle believes that there is also a chemotaxic phenomenon between bacteria and different tissues. He says: 'In no other manner can the localizations, often very specific and constant for a given bacterial species, be explained.' [18]

B. PHAGOCYTOSIS

After the leukocytes have been attracted to the site of the irritating substances produced by the organism, the same chemical and physical processes continue The lowering of the surface tension of the leukocyte causes a complete surrounding of the organism by the protoplasm of the cell. After the organism has been taken up, it may be killed and become disintegrated, falling prey to the intracellular digestive processes of the leukocyte.

The digestive principles of the leukocyte are hard to isolate. Probably the only place in which the action may be manifested is within the interior of the phagocyte. Bactericidal substances may be found in extracts of leukocytes, and protease may be found in extracts from sterile exudates, [19] but Jochmann [20] believes that living bacteria are not susceptible to this enzyme; and it may be that the organisms are killed within the phagocyte by a different mechanism from the one by which they are later digested. D'Herelle [21] mentions four different ways in which the bacteria can resist phagocytosis; (1) by repelling the leukocytes;

(2) by forming a capsule; (3) by neutralizing the digestive fermen the leukocytes; and (4) by digesting the leukocytes.

In many cases, organisms remain within the leukocyte wit affecting it any more than carbon particles do—that is, the cell conti its normal existence and the organisms do not multiply to any de; The cell may then have its surface tension altered in such a way th moves away from the site of original attraction, again passes througl capillaries or lymphatics into the circulation, and is carried to other p of the body. Or it may be lodged in the neighboring lymph glands. T bacteria may thus become metastatic and, upon the later death of leukocyte, may produce a new focus of infection. On the other h these loaded leukocytes may be taken up by other larger phagocytes the bacteria be destroyed by them.[22]

The phagocytosis of bacteria has been studied in test-tube experime and it has been found that washed leukocytes have very little powe phagocytosis, but in the presence of normal or immune serum they once become active. Immune serum contains substances that make leukocytes much more active in phagocytosis than does normal ser This links up the cellular phenomena of immunity with the hum phenomena, which will be considered below.

II. THE ROLE OF THE FIXED TISSUE CELLS IN IMMUNIT

Certain of the fixed tissue cells of the body take part in the destruct of bacteria. They react in very much the same way as wandering c except for the limitation of their movements. For the most part, they held by the cement substances and fibers binding them to neighbori cells. But it is believed that at times the endothelial lining cells of blo vessels may detach themselves and wander off in response to a ve powerful chemotaxic influence.[23] Certain it is that these cells, both capillaries and in the immediate vicinity of them, have been seen phagocyte bacteria and other foreign substances. This is particularly tr of the lining cells of the body cavities—the peritoneum, the pleura, t joints, and Von Kupffer's cells of the sinusoids of the liver. The only ce of epithelial origin that have the power of phagocytosis appear to be tl epithelial cells of the lung alveoli and the epithelial cells of the conjun tivae.[24, 25] The invisible role of some of the fixed tissue cells in tl destruction of bacteria has not yet been finally determined. Aside fro phagocytosis, it is believed that certain of the fixed tissue cells of tl body produce the antibodies that act upon the bacteria through tl medium of the body fluids. Particular interest has been focused recentl upon the reticuloendothelial system.[26] It is believed that whatever cel are responsible for antibody production are altered by the immunizin

process in such a way that for a long time after all demonstrable antibodies have left the blood, the cells can rapidly mobilize certain defensive elements with which the body resists infection.

It should also be called to mind that the fixed tissue cells, as well as the intercellular substance, produce in many cases an effective physical barrier against the spread of bacteria. This is true, also, for the reparative tissue that makes its appearance very early in the process of inflammation

III. THE ROLE OF THE BODY FLUIDS IN IMMUNITY

The work of Von Behring [27] in demonstrating that the serum of animals immunized against diphtheria and tetanus contained a substance that effectively destroyed the pathogenic power of diphtheria and tetanus bacilli, brought to light the fact that, for certain infections at least, the cells were not important in the body defense. It was found, however, that the serum was effective not against the organisms but against the soluble toxic product of the organisms In other words, the serum contained a substance that very definitely neutralized the effect of the soluble toxin. This finding was followed by the experiments of Pfeiffer,[28] who demonstrated that cholera vibrios injected into the peritoneal cavity of guinea pigs vaccinated against the cholera organisms became progressively granular and finally dissolved; whereas in normal animals, they multiplied and finally killed the pig. If immune serum were injected into a normal guinea pig, the organisms subsequently injected were killed in the same manner as in an immune pig. In other words, the immune serum carries over certain substances that either directly cause a dissolution of the bacteria or enable some mechanism that has the same effect to be initiated within the normal pig. This protective substance in the serum of animals immune to cholera was seen to be quite different from the immune substance against tetanus and diphtheria, and demonstrated for the first time a bactericidal or bacteriolytic substance. Then followed the discovery of certain phenomena recognized as specific properties of immune serum. At first, each of these properties was considered to be the function of an integral substance. These were called by different names according to their demonstrated action: agglutinins by Gruber and Durham,[29] precipitins by Kraus,[30] opsonins by Denys, Wright, and Neufeld,[31-33] hemolysins by Bordet,[34] and later lytic antibodies for other types of tissue cells.

A THE ROLE OF THE BODY FLUIDS IN NATURAL IMMUNITY

The body fluids of normal animals contain certain substances corresponding in their reactions to the antibodies of immunized animals, but they are not present to high degree, and their action is weak They differ



FIG. 5 PFEIFFER Dr. Hermann Pfeiffer demonstrated the value of producing active immunity by means of vaccination, and the development of bacteriolysins in immune serum. (From *History of Bacteriology* by William Bullock, Oxford University Press, 1938)

somewhat from the antibodies of immunized animals in their physical characteristics, thermostability, and specificity, and they probably play a very small part in natural resistance to disease, the biggest factor in natural resistance being the phagocytic power of the leukocytes, as has been stated above. The antibodies in so-called normal animals are of

doubtful origin. Some may be inherited and others may be received from the mother through the placental circulation by the fetus. Ehrlich[35] found that immunity to ricin and abrin was transmitted to offspring from the mother but not from the father. On the other hand, it is quite possible that these antibodies may be derived by the absorption of small quantities of bacteria or of bacterial products from the intestine or from previous mild infections. As mentioned above, natural immunity, unlike acquired immunity, cannot be passively transferred from one animal to another.[36] The fact that normal agglutinins are not present in children but are found in adult serum would seem to indicate that at least some of these antibodies are not really natural, in the sense that they are normally present, but that they have been acquired in the course of time. Landsteiner has pointed out that natural antibodies are more resistant than those resulting from immunization.[37]

B. THE ROLE OF THE BODY FLUIDS IN ACQUIRED IMMUNITY

By far the greatest amount of our information in regard to immunity has come from the study of the changes brought about in the serum of animals by the injection of foreign proteins. The phenomena of antibody formation is not limited to bacteria or to bacterial derivatives. It is of much more general biological significance, and we cannot say that it is a mechanism of nature for the purpose of protecting the body against infectious disease.[38] The injection of foreign proteins is followed by alterations in the serum of the injected animals. Although we do not know the nature of the substances producing these alterations, we may recognize the alterations by certain laboratory tests. We call the injected substances antigens because they have the power of generating antibodies that react both chemically and physically to them. Antigens are of two types: first, those that are primarily toxic and enzyme-like; and second, those that are nontoxic and nonenzyme-like.[39] Correspondingly, there are two types of antibodies. Those formed in response to the toxic antigens are called antitoxins and have certain relatively simple reactions, which will be discussed below. The second form a large group the members of which have been given different names according to the different phenomena for which they are responsible. They include agglutinins, precipitins, bacteriolysins, opsonins, and complement-fixation antibodies. These also will be discussed in more detail below.

IV. THE NATURE AND PROPERTIES OF ANTIGENS

Antigens are soluble and nondiffusible, and can react with the cellular elements of the body only by cellular surface relations. They cannot enter the cells. All soluble proteins are antigenic, but if they become coagulated



Fig. 5. Pfeiffer. Dr Hermann Pfeiffer demonstrated the value of producing active immunity by means of vaccination, and the development of bacteriolysins in immune serum. (From *History of Bacteriology* by William Bullock, Oxford University Press, 1938)

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they lose their antigenic power; because, in so doing, they lose their solubility and their power of coming into contact with living cells. The protein molecule is so complex that it is difficult to determine whether the whole or a part of it is chiefly responsible in the stimulation of an antibody. Proteins are made up of combinations of about twenty amino acids, which may be arranged in many different ways within the protein molecule. Inasmuch as there can be several quintillion combinations of these amino acids, proteins may differ markedly in their make-up.[16] Thus, it is not difficult to understand that antibodies stimulated by these many different forms may be specific and react only to the antigen that stimulated their production.

Wells[40] points out that the specificity of immunological processes is simply another instance of biological specificity, as exemplified in the fertilization of the ovum by the sperm of a given species. Similarly, growth processes produce specific forms and functions and govern the repair of tissues after injury. Wells states: "'The entire protein molecule is probably not involved in determining the specific character of the immunological reaction, but this is developed by certain groups or radicals of the protein molecule and a single protein molecule may contain two or more such groups.' It is probable that the aromatic radicals of the protein molecule are the groups concerned.[42] Different antigens vary in the amount of antibody they stimulate, and different animals vary in the amount of antibody they produce.

It is obvious that in the human body are many different proteins, and no one of them can be called the specific human protein. In human blood serum, for instance, there are four or five different proteins, and in egg white, at least three.[41] On the other hand, there are within the human body certain proteins more like similar proteins in other species than like the other human proteins of the serum or tissues; for instance, the human lens proteins, of which there are two, are similar to the lens proteins from all other species, and the casein of human milk is indistinguishable from the casein of the milk of animals.[43] It has been claimed that certain lipoid substances have antigenic power, but Wells believes that there is probably some protein fraction attached to these, for lipoids readily take up protein.[44]

Landsteiner[45] attacked the problem of determining what portions of the protein molecule are responsible for antibody production. He made synthetic additions to the protein molecule, showing that specificity may be altered. By making many substitutions, he found that the specific behavior is determined by the chemical structure of small portions of the large antigen molecule.

From an antigenic point of view, Zinsser[46] said, 'infectious diseases may be looked upon as an invasion of the body by a living foreign pro-

tein, which must be assimilated and disposed of.[7] Bacteria represent relatively simple antigens, and for the most part can be considered to have a fairly constant antigenic power. Moreover, the antigens in some species of bacteria resemble very closely the antigens of other bacteria of the same morphological groups. For example, the antigens of all typhoid bacilli are similar to each other and show some similarity, also, to the antigens of the bacilli of the paratyphoid, colon, and dysentery groups. On the other hand, the antigens of the streptococci differ markedly within that group, so that for certain of the antibody phenomena each strain differs to some extent from every other strain. In general, the antibodies to bacteria are specific, but certain bacterial antigens produce a nonspecific immunization; and Eberson[47] has obtained evidence that different species of bacteria may contain common antigenic proteins accounting for group reactions and nonspecific immunization.

Wells brings out another interesting point concerning the specific action of antigens, namely, that animals previously immunized against one type of bacteria are more capable of forming antibodies for some entirely unrelated organisms than are control animals, and persons who have once had typhoid but whose blood no longer contains the agglutinin antibody may later demonstrate in the serum a very high agglutinin content when infected by some other organism or even after any sharp febrile attack.[48] This indicates some change in the cells permitting quick mobilization of an antibody after it has once been produced.

V. THE NATURE AND PROPERTIES OF ANTIBODIES

As has been said above, the cells of the body, aside from the phagocytes, have presented to them by the circulation, for their metabolism, chemical substances that have been digested down to very simple products Being highly differentiated, they are unable to absorb directly or modify such complex chemical substances as proteins. When bacteria and certain of their products come into contact with the tissues, they represent complex chemical substances. The phenomenon of antibody formation against bacteria or other foreign protein constitutes a mechanism for the disposal of this foreign protein If the foreign protein is in a living state and increases by multiplication, then the problem of its disposal becomes much more complicated. Inasmuch as these substances cannot enter the cell, if they are to be digested at all, the digestive ferments must be manufactured either on the surface of the cell or within the cell and later be cast off into the surrounding tissue spaces. If they are cast off from the cell, they are taken up by the circulation and they are then to be found free in the plasma of the blood or in the serum after the blood has clotted. The antibodies appearing in the blood of immunized animals represent

atom complexes normally parts of the body cells and concerned in their metabolic processes.' [49]

When antibodies are produced in response to the presence in their neighborhood of, or by contact with, antigens, following the general law of overcompensation, they are overproduced.[49] For instance, the amount of antitoxin in an immunized animal is out of all proportion to the amount of toxin injected.[50] Horses immunized to tetanus or diphtheria may be bled again and again and the antitoxin will continue to be thrown out into the regenerated blood.[51] These facts favor the opinion that the antibodies of the serum are produced by the body cells.

It is not known just what cells of the body produce these antibodies. The spleen, the bone marrow, and other tissues have been variously considered. They are still produced, however, when the spleen is removed, and the resistance of animals and man to infections with bacteria is not noticeably diminished by splenectomy.[52] Wassermann and Citron [53] demonstrated that the injection of rabbits with typhoid bacilli intraperitoneally, intravenously, and intrapleurally showed a greater content of antibody in exudates from the tissues into which the original injection was made than in the blood serum, thus apparently demonstrating that antibodies are produced by the cells with which the antigen comes into most intimate contact. However, it has been demonstrated that antitoxin is not formed by the tissue upon which toxin has its most powerful action; for, as d'Herelle has observed, animals immunized to tetanus have no greater content of antitoxin in the cells of the central nervous system than elsewhere, and if tetanus toxin be injected into the central nervous system, these animals will die of tetanus.[54]

One of the most striking and important characteristics of antibodies is the fact that they are specific, that is, when a given antigen is injected, the phenomena that take place indicate that the substances formed in the body and present in the serum will react only with the original antigen. This general rule has certain exceptions, which will be referred to elsewhere.

As has been stated above, it cannot be said that antibody formation is strictly a protective mechanism of the body against bacterial disease, because it is a general biological phenomenon for all protein substances. By the same token, we cannot say that all of the phenomena of antibody action are beneficial to the host and deleterious to the bacteria. In fact, although we speak of these phenomena as evidences of immunity, they may not represent any real protection of the body against disease. This is particularly true of the phenomena of agglutination and precipitation; and it is quite possible that there are other immune substances not demonstrable by test-tube experiments but really of greater importance in the immunity to disease than are certain of the antibodies that are

demonstrable. The consideration of the behavior of antibodies will be discussed under their common names.

A. Antitoxin

Antitoxin is a substance in the serum of an animal that has been inoculated with the filtrate from a culture of the bacteria that produce true toxin. The organisms with this property of producing true toxin have been mentioned in Chapter vi. Suffice it to say here that the organisms of prime importance from this standpoint are the bacilli of diphtheria and tetanus and, to a lesser degree, the anaerobic organisms of the gas-forming group. There are, also, certain vegetable poisons, of which ricin is the most common example, and certain animal toxins such as snake venom and spider poison. The filtrate from the cultures of the organisms of diphtheria and tetanus have profound toxic action on many species of animals; but when the serum of an animal immunized to small doses of the toxin is injected in sufficient quantities into normal animals, before, with, or after the toxin, it completely prevents the intoxication of the animal. The earliest work in this sphere was accomplished by von Behring and Kitasato[27] and Roux and Vaillard.[65] It was first thought, and it was maintained by Ehrlich, that the union of toxin and antitoxin followed the law of multiple proportions, and that antitoxin would neutralize just so much toxin and leave the rest unchanged.[56] Later Arrhenius and Madsen[57] came to the belief that the reaction of toxin to antitoxin was similar to that between weak acids and weak bases, following the law of mass action. It was soon found that this was not the case, but that relatively small quantities of antitoxin could reduce the toxicity of a large quantity of toxin, much as a small quantity of dyestuff will be taken up faintly but evenly by a fabric dipped into it. Bordet[58] maintained that the antitoxin molecules distributed themselves equally upon all the toxin molecules present, partially saturating and attenuating them, but not uniting with some and leaving others entirely free. Toxin and antitoxin do not form a strong chemical union, and inasmuch as they vary in their heat stability, heat may destroy one and leave the other intact. The combination may also be dissociated by acid[59] or filtration.[60]

The toxins of diphtheria and tetanus have a very definite affinity for certain tissues of the body and form a firm union with these tissues. When antitoxin is present in the blood, upon the introduction of tetanus toxin, the antitoxin combines with the toxin in such a way as to divert it from the cells of the central nervous system. If, however, union has already taken place between the toxin and the tissue of the central nervous system, then the antitoxin will not withdraw or remove the toxin from this combination. It is particularly interesting to note that, as has been mentioned above, the central nervous tissue cells of susceptible animals will

absorb the tetanus toxin, but other tissue cells in susceptible animals, such as the liver, spleen, and kidney, *cannot do so, nor* can the central nervous system cells in nonsusceptible animals.[61]

Just what cells of the body produce antitoxin is not known. It is obviously produced by some cells which come in contact with the toxin and attempt a parenteral digestion of it, but it is probably not produced by the cells for which the toxin has a special affinity, as has been mentioned above. The selective action of toxin is analogous to the selective action of certain known chemical substances—to alkaloids, narcotics, and other drugs; and we must assume that there is either a chemical or a physical affinity between the toxin and the cell.[62] This selective action of bacterial poisons must be distinguished from the selective localization of certain bacteria in certain tissues; for example, the diphtheria organism localizes in the mucous membrane of the nasopharynx, but the localization of its toxin is largely in the tissues of the central nervous system

B. THE ANTIBODIES TO THE NONTOXIC ANTIGENS

The antibodies to the nontoxic antigens are put in a single group, for they may all be one and the same substance, showing different properties under different physical conditions. These properties were observed at different times and in different circumstances, and it has been thought that the substance responsible for each function was in fact a different antibody. It is now thought by many observers, considering the subject from a broader point of view, that all of these functions simply represent different properties of a single antibody. This attitude was maintained strongly by Zinsser,[63] who said in effect that when antigen and antibody unite, the phenomena that take place depend upon the physical state of the antigen, the nature and presence of certain co-operative substances, and the environmental conditions under which the observations are made. In other words, when the antigen is particulate matter, such as a bacterial cell, the union with the antibody is made visible by agglutination in the presence of certain electrolytes, when it is in soluble form, the phenomenon is visible as precipitation; when phagocytes are present, we see phagocytosis; when the antigen is bacteria or tissue cells amenable to the lytic process in the presence of a complement, we have bacteriolysis or cytolysis.

The chief objections to this 'Unitarian doctrine,' as it is called by Zinsser, are that there is not a parallelism in the visible reactions as represented by these different functions; and that certain antibodies seem to be absent entirely when others are present. For example, with the pneumococci there are strong agglutinins in the sera specifically 'immune' to any of the three main groups of pneumococci, but there are effective protective antibodies only in the serum immune to Type I, II, and XX.

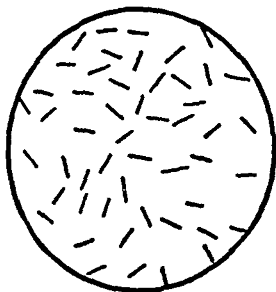
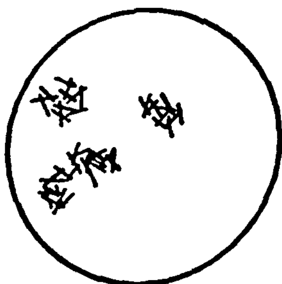


Fig. 6 Microscopic agglutination reaction (From Dr. F. P. Gay, *Agents of Disease and Host Resistance*, Thomas, Springfield, Illinois, 1935)



respect it is similar to other antigen-antibody complexes, which will be mentioned below. Although the property of agglutinating bacteria probably has a relatively slight function in the defense of the body against infection, the phenomenon is of real, practical value in the identification of organisms. The most striking examples of this are the separation of the various types of pneumococci and the members of the typhoid, paratyphoid, and dysentery groups.

Agglutination is not perfectly specific—that is, when a given organism is injected into the body for the purpose of producing immune serum, this serum, after the proper time for the development of the antibodies, will agglutinate not only the organism used for the immunizing process, but also, to a certain degree, organisms of a similar antigenic nature. Thus we have group agglutination, with major and minor groups, and this may take place not only within the species but also outside it, as for example, the reaction of the serum of patients with typhus fever to *Bacillus proteus*[69] and the agglutination of the colon organism by patients with typhoid fever. Zinsser says: 'For practical purposes, the diagnostic value of specificity is not affected, since the action of minor agglutinins can always be easily eliminated by sufficient dilution.'[70] This is questioned by Krumwiede et al.,[71] for they have found that related species of organisms will frequently react more strongly than the homologous organism used for producing the serum. In a study of cultures from an epidemic of puerperal fever, the author demonstrated a splitting of the two phases of the agglutination phenomenon.[72]

When bacteria are injected into an animal and agglutinating substances are formed in the serum, it has been found that the earliest evidence of agglutinin is the action upon the organism injected; but with a continuation of the injection, other strains of the same type are agglutinated to a certain degree, and if the injection is continued indefinitely, large larger groups are included.

Agglutinins may be present not only for bacteria but also for other Of particular importance is the agglutination of red blood cells of species in the serum of another species, or even in the serum of ano individual of the same species. These phenomena will be discussed Chapter xvii.

2. *Precipitins*

The precipitation of protein in serum is similar to agglutination. S called colloidal solutions are really suspensions of particles all having th same electrical charge and therefore not dissociable. Precipitation take place under certain circumstances in which the electrical charge of these particles is changed.[73] Precipitation, then, refers not to the bacteria themselves but to their products; just how much of a factor it is in the

actual process of immunity is not definitely known. It is probable that when the antibodies are fixed by union with the bacterial products in colloidal solution, these deleterious substances may be diverted from their action upon body cells by precipitation. Just as with agglutinins, precipitins first form with homologous proteins, but if immunization continues, the phenomenon embraces closely related species in a wider and wider field.[74]

3. *Lytic Antibodies*

Certain bacteria and other cells are apparently directly destroyed by contact with immune serum. Pfeiffer[75] showed that cholera vibrios injected into the peritoneum of immune guinea pigs very promptly became disintegrated. It was then found by Bordet[76] that the serum of these immune animals contained a substance that would have the same effect upon the organisms without the possibility of cellular collaboration. He also showed that heated serum had no effect but could be reactivated by the addition of normal serum, which of itself had no effect. Bordet thus demonstrated that there are two steps in the lytic process: one, a combination of the antigen with the specific antibody present only in immune serum and heat-resistant, and second, the destruction of the antigen by a heat-labile substance present in normal serum. This substance has been called *alexin* or *complement*. Further studies of these phenomena led to a clearer conception of antigen-antibody union by Bordet, which is now generally accepted, for it was found that in the absence of the antibody the complement would not destroy the antigen. This discovery led Ehrlich[77] to believe that the antibody acted as a link between the antigen and the lytic substance, but it was later found that neither the antigen nor the antibody alone can unite with the complement. That is, neither the antigen nor the antibody alone has any binding power for the complement. Therefore, the complement can be fixed only when the union of antibody and antigen has taken place.

The practical importance of the bacteriolytic power of immune serum in the general process of immunity is not clearly understood. Bacteria apparently differ greatly from one another in their resistance to the lytic action of immune serum. Cholera vibrios are particularly susceptible, whereas the streptococci are particularly resistant. This may be due either to a chemical or to a physical difference in the bodies of the bacteria. The bactericidal substances of leukocytes are quite distinct from the lytic substances of immune serum, and are independent of the participation of this lytic substance.[78]

4. *Opsonins*

The degree of phagocytosis of bacteria by polymorphonuclear leukocytes and other phagocytes is greatly enhanced by certain substances in immune serum and designated by the term 'opsonins' Opsonins are present to some extent in normal serum. Leukocytes freed from serum have very little power of phagocytosis, but in the presence of serum, particularly immune serum, they are very active in taking up certain bacteria. With this phenomenon, also, there are apparently two stages: first, a union of the antibody with the antigen, and second, an ingestion of the antigen-antibody complex by the leukocyte. The antibody is not destroyed by heat, but the lekocytes cannot, of course, carry out the function of phagocytosis if they have been injured or killed. This phenomenon has been studied very extensively by Wright,[79] and he has used it to measure the degree of immunity to those infections in which the phagocytosis of the bacteria seems to play an important role, as, for instance, in staphylococcus infections. He has shown that the resistance of animals to staphylococcus infections runs parallel with the power of their leukocytes to phagocyte these organisms, and he has found that the leukocytes of patients who are in a state of infection by these organisms have very little phagocytic power. As immunity increases, the phagocytic power correspondingly increases. Unquestionably the factor of phagocytosis is of prime importance in natural immunity, in acquired immunity it probably plays a secondary role [80] It is a very important element in the resistance of the body to disease processes, as well as in the struggle between the body and the invading organism when once the latter has gained a foothold in the body. However, as has been stated above, organisms may be phagocyted and still resist digestion or destruction by the bacteria.

5. *Complement-Fixation Antibodies*

Complement-fixation antibodies apparently do not differ in any way from the antibodies that play the role in the phenomenon of bacteriolysis, for there the complement is fixed by the antigen-antibody complex. Such fixation can be made visible only when another antigen-antibody complex is added to the first. For example, sensitized sheep cells become hemolyzed if complement is available, but remain nonhemolyzed if the complement is first fixed by previous contact with another antigen-antibody complex. This phenomenon has given rise to many different laboratory tests, chief of which is the Wassermann test. For the most part, these are relatively unimportant from the standpoint of surgery and will not be discussed further in this place.

VI. THE ROLE OF CHEMICAL VARIATIONS IN IMMUNITY

There has been a great deal of study of the selective action of bacteria for different tissues of the body, but so far there has been no adequate explanation of this selectivity beyond the general statement that it is due to some chemical or physical affinity between the bacteria or their products and the tissue cells. This affinity is quite distinct from the accidental contact established by the entrance of the bacteria into the body by different routes. As we have said above, certain bacteria may enter the body in one place and be destroyed, whereas if they enter at another place they will cause disease. When once the organisms gain entrance into the physiological interior of the body, they apparently differ greatly in their invasive properties. It is quite possible that those passive factors of circulatory currents—passive so far as the bacteria are concerned—act the same with all organisms, but certain organisms are never heard from again, whereas others produce metastatic foci of infection. The first are destroyed in the blood, or as they run the gauntlet of the capillaries are phagocyted, or are carried to tissues in which they are either destroyed or live without causing any disturbance. For the most part, they are probably killed. We say in general terms that it is because they meet unfavorable environment and are unable to adapt themselves to it quickly enough to survive. One factor may be the chemical variation represented by differences in hydrogen-ion concentration in various tissues of the body. Rous [81] a number of years ago performed some very interesting experiments that indicate that various tissues of the body differ markedly in their hydrogen-ion concentration. We know that in artificial media this is a factor very important in either favoring or inhibiting bacterial growth.

In unfavorable tissues, it may be that the bacteria are simply unable to find the necessary food requirements or are unable to modify by digestive processes the complex chemical substances around them, or they may meet active digestive processes on the part of the tissue cells.

VII. EXTRINSIC FACTORS MODIFYING RESISTANCE TO INFECTION

A number of extrinsic factors may operate through the medium of the body to increase or decrease the resistance to infection. One of these factors recently brought to greater prominence is diet. It has been known for a long time that diabetics are susceptible to infections, and it is supposed that this is due to the higher level of sugar in the tissues as well as the interference of blood supply by arteriosclerosis. Even if there

is no real diabetic state it is a common observation that those prone to recurrent skin infections with the staphylococcus frequently have such a recurrence following the consumption of an unusual quantity of sugar in the form of candy.

Whipple[illegible] has shown that in the presence of infection, a patient will lose protein and go into a negative nitrogen balance unless the intake of protein is greatly increased. Frequently, as Van Slyke has said,[illegible] 'It is impossible to correct a negative nitrogen balance and maintain a favorable balance in the presence of infection by the intake of protein in the food or the parenteral injection of amino-acids.'

The association of vitamin deficiency with infection has been studied by many investigators, and evidence seems to be available that Vitamin A lack increases susceptibility.[illegible] The other vitamins apparently play no role in resistance. Animals fed on diets deficient in Vitamin A are much more likely to acquire spontaneous infection and succumb to experimental inoculations than are animals fed on normal diet.[illegible] Shibley,[illegible] on the other hand, failed to confirm these results. It has been found, in any event, that an excess of this vitamin will not increase resistance above normal.

Mills[illegible] has shown that his rat colonies kept at tropical heat need a higher intake of vitamins, particularly thiamin and cholin, in order to resist infections as readily as the rat colony kept at room temperature.

The seasonal incidence of certain infections has, of course, suggested that heat and cold and changes in humidity alter the body resistance to infection. A very frequent item in the history of cases of staphylococcus septicemia is that the patient was exposed to cold at the time the local infection (furuncle or carbuncle) became general. Hemolytic streptococcal infections are prevalent in temperate zones in the late fall and late winter when fluctuations of atmospheric temperature around the freezing point are most frequent. Probably, at these times the dose of organisms taken in by the individual is unusually large and thus plays a greater role in the initiation of the infection than do any changes in the body of the patient. Chilling, however, causes a congestion of mucous membranes and permits organisms present on the surface to become imbedded in the tissues, and if the congestion is not relieved in a few hours, infection may become established.

Some have been led to believe that the effect of long daylight in the summer months, with the prolonged hours in which ultraviolet radiation is available, is a major factor in lowering the incidence of infection. It is explained not by the lethal action of these rays upon the organisms, but by the development of peroxides in the tissues subjected to the radiation. Experimental evidence is lacking, however, to prove that ultraviolet radiation actually increases the resistance to infections of animals or man.

VIII. THE FACTS OF IMMUNOLOGY AS APPLIED TO THE SURGICAL ORGANISMS

The presence of antibodies can be demonstrated in the blood after recovery from many of the medical infections, and even after they hav disappeared, the immunity lasts. In many of the exanthemata, one attac renders the patient permanently immune to later attacks. On the othe hand, against the necrotizing and pyogenic organisms, which cause a local infection with local destruction of tissue and the collection of purulent exudate, namely, against the surgical organisms, human and other animal cells cannot produce any demonstrable antibodies in the serum, or any lasting immunity. This statement applies particularly to the hemolytic streptococcus, the hemolytic *Staphylococcus aureus*, the colon bacillus, the proteus group, *Ps. pyocyanea*, and the clostridia of gas gangrene or tetanus. Furthermore, any attempt to inoculate animals and thereby produce in their serum antibodies that could be passively transferred with benefit in the presence of human infection has been extremely disappointing and unsuccessful.

It is true that certain of these organisms produce true soluble toxins, which will call forth antitoxins, but these are not effective in the presence of highly invasive organisms. They are of major importance, of course, in tetanus and gas gangrene but of distinctly minor importance in staphylococcal and streptococcal infections.

IX. LOCAL IMMUNITY

Surgical infections are local infections and recovery from them, although it is greatly aided by surgical drainage, must take place by overcoming the infection by the local tissues. For this reason, the surgeon should be greatly interested in the possibilities of natural and acquired local immunity.

We have mentioned above the importance of the portal of entry for bacteria, and we have shown that although a few bacteria may penetrate the body and gain a foothold at almost any point, most bacterial species can gain a foothold only under certain circumstances and in certain places; and almost all organisms find a difference in the resistance of various tissues to the establishment of their growth and multiplication. Thus it is obvious that aside from the mechanical variations making it easy or difficult for organisms to enter the tissues, when once they have entered, the tissues themselves vary considerably in their ability to forestall or overcome bacterial growth and the development of an infection. This may be considered as a local difference of resistance, and where

certain tissues are entirely resistant to the infection of certain organisms, we may say that those tissues are immune to the infection But within recent years, more and more evidence has been brought forward to show that tissues not only differ in their natural resistance but that they may also, in fact, acquire an immunity that they did not have before, in which the other tissues of the body and the body as a whole do not share. For example, when a virulent streptococcus infection comes to a standstill we must assume that something has happened within the body to make the body victorious in its struggle against the invading organism. It is not possible in such circumstances to demonstrate any general immune substances in the serum, but there must be a local acquired resistance.

In the natural resistance of tissues to the establishment of an infection a number of factors are involved, some extrinsic and others intrinsic in the tissue cells. Of the former may be mentioned the structural density of the intercellular substance and the richness of the blood supply of the part. This last factor has been mentioned frequently as being of considerable importance in the resistance to infection of the tissues of the scalp and face, as well as certain of the vascular internal organs. It is conceivable that the blood supply may be directly responsible for the increase of those antibacterial elements brought from other parts of the body to the place where the bacteria have entered—in other words, the wandering phagocytes of the blood and the antibodies of the blood plasma. On the other hand, it is probable that the blood supply also acts directly by bringing to the specific tissue cells elements that they in turn can utilize for their own offensive action against the bacteria.

The acquired immunity of a tissue is believed to be something intrinsic. It has been studied intensively by Besredka,[88] and although the evidence is meager, still for certain organisms and for certain tissues there seems to be very definite evidence that tissues may by direct contact with micro-organisms build up an immunity that they did not have before. Gay[89] summarized this whole question of local immunity and added to the results of Besredka, certain findings of his own, which seem to bear out this contention. As Gay understood it, local immunity represents a superior mechanism for the disposal of a particular micro-organism rather than a local mobilization of antibodies generally present in the body. 'Local immunity may be proved by the local presence of antibodies before their appearance elsewhere in the body or by their local presence in greater concentration.'

In the very early days of the study of immunity, Pasteur[90] found that he could immunize sheep by feeding anthrax spores, though with that organism he found such immunization inferior to subcutaneous inoculation. Besredka[91] found that typhoid immunity may be built up better



FIG 7 BESREDKA Dr. Alexandre Besredka was particularly interested in the problem of local immunity. (Courtesy of New York Academy of Medicine)

by intestinal vaccination than by subcutaneous inoculation, and suggests that there may be a general biological law that indicates that the best site for the development of local immunity is the natural portal of entry for the organisms.

Local immunity to streptococcus was first noted by Fehleisen.[92] He inoculated human beings with erysipelas and then found that the areas previously covered by the disease were immune to secondary injections.



FIG. 8. LEVADITI. Dr. Constantin Levaditi demonstrated the specificity of local immunity. (Courtesy of New York Academy of Medicine)

It was later found by Meierowitsch[93] that rabbits were locally immune to the streptococcus of erysipelas for from one to two months. This has been confirmed repeatedly, and these areas are found to have local immunity when the other tissues of the body and the blood serum show no evidence of protection or antibody formation. In clinical cases during the First World War, Levaditi[94] found that wounds which had been infected with streptococcus and subsequently became free from these organisms would later kill large numbers of the same strain planted

directly upon the wound, but these wounds showed less resistance to other strains, indicating that there was a certain degree of specificity in the immunity. The resistance of local tissues to infection can be increased nonspecifically by the injection of certain other substances, for example, plain broth medium, but this resistance is not as great as the immunity produced by the injection of the organism itself or the products of the organism.[95, 96] Besredka [97, 98] found that when the filtrates of streptococcus and staphylococcus cultures are applied to the skin of guinea pigs, the local areas are protected against subsequent infection by the same organism.

Microscopic examination of the locally immune tissues has indicated that an anatomical change may be responsible for the development of local immunity. This is particularly true when body cavities like the pleura and peritoneum are considered; for the exudates that occur in response to the infection are poured out into the cavity and are made more easily visible than the exudation into the cellular tissues.[99] Metchnikoff observed large mononuclear phagocytes but believed that they played only a minor role in the removal of bacteria, except in chronic forms of inflammation.[100] Gay believed that these cells, which by most observers are now termed 'clasmatocytes,' have a very important role to play in the development of local immunity.[88] Sabin [101] and others believe that they come from the same ultimate source as the large mononuclear of the blood and the endothelial cells of the blood vessels.

If these cells play the chief role in local immunity, we must call them extrinsic rather than intrinsic factors. Furthermore, immunity so produced would be a local manifestation of a general body immunity, particularly if these cells come from elsewhere in the body via the blood stream. It is probable, also, that if these cells were alone responsible, the tissues themselves would not differ in the degree of local immunity. The fact that such a difference does exist would seem to indicate that the specific tissue cells have something to do with the development of local immunity, even if it is but a secondary role. If these cells arise in situ from the endothelium of blood vessels, they do not represent a specific tissue cell reaction but a local nonspecific reaction. From either standpoint, the vascularity of the part would be an important factor, either by providing an increased number of those cells from the reticuloendothelial system or by carrying the cells from their places of production elsewhere.

Clinically, it has always been an interesting observation that the tissues around the anus rarely become infected with putrefactive and anaerobic intestinal organisms after operations for hemorrhoids or fistula, although elsewhere these organisms frequently cause the foulest infections, as in wounds made for the drainage of appendiceal abscesses. An attractive theory, as yet unproved, is that there is a certain local immunity of the



FIG. 9. GAY Dr Frederick Gay's studies in local immunity led him to the belief that the clasmatocytes played a leading role in this phenomenon. (Courtesy of Mrs. Gay)

tissues in the anal region as a result of frequent invasion by small numbers of these organisms.

It has been repeatedly observed that secondary operations on the peritoneum are infinitely safer than primary operations. There is definitely less tendency to peritonitis, even after gross contamination of the cavity. It may well be that this is a feature of local immunity. This very important phenomenon requires careful study.

The role of local immunity in the development of immunology is one that is destined to have an active development within the next few years, and its interest is chiefly stimulated by the work of Besredka and Gay.

X. WHAT CONSTITUTES LOWERED RESISTANCE

As has been stated above, in the great majority of instances when bacteria enter the body, they fail to produce disease. They must enter by the proper route, and in sufficient quantity, and be virulent enough to overcome the body defenses at the point of entrance, in order that they may gain a foothold, grow locally, and produce the local and general signs and symptoms of disease.

But while a given quantity of virulent organisms entering the body by the usual route will in some individuals produce disease, it will in others fail to do so. We have considered the defenses of the body and in what manner they may be increased, but we have not considered under what circumstances these defenses may be absent or inhibited. It is a frequent clinical observation that in certain cases the patient is overwhelmed by an infection that gives no evidence of the point of entrance and which causes death in two or three days. We call this a fulminating infection, and say that the patient had no general resistance to the causative organism. It is probable that in such cases there is an absence of local immunity at the point of entrance, and the products of bacterial growth and their action on local tissues or on vital centers effect a fatal outcome before the defensive elements of the body can be mobilized. In children, a similar absence of resistance seems to exist locally, although there is often a general resistance that prevents death. The organism produces no reaction at the point of entrance and is not heard from until some internal focus, such as an osteomyelitis or an endocarditis, gives symptoms. In such instances, the organism may not be very virulent, but the body has no local resistance at the point of entrance to hold it there even temporarily.

A local process at the point of entrance indicates a certain resistance to the organism. At times, a very violent reaction at the point of entrance may indicate a hypersensitive state that represents an early stage of immunity.

Infectious diseases are more likely to develop in those individuals whose bodies have been subjected to severe trauma, to starvation, to exposure, or to chilling than in normal individuals. These conditions do not particularly favor the invading organisms, but they inhibit the various defensive mechanisms of the body, either locally or generally. Persons suffering from severe acute infections, such as measles or influenza, are more subject than normal individuals to a secondary invasion of organisms like the streptococcus. Persons with chronic diseases affecting the quality of the blood, as in the various anemias, or affecting the quantity of the blood delivered to the tissues, as in the various forms of heart or

blood-vessel disease, are more likely than normal individuals to develop infections. Individuals suffering from disorders of metabolism, such as diabetes or nephritis, are likewise more subject to infectious diseases than are normal persons.

What is the nature of this lowered resistance? It is probably not simple in most cases. In certain conditions, obviously, the primary difficulty is the maintenance of the cellular nutrition; in other conditions, it is the failure to mobilize the defensive mechanisms; and in a few instances, the local conditions probably favor the growth of the invading organism. In some instances, in clinical cases, and frequently in laboratory animals, it is found that in debilitated states there is deficient complement or alexin in the blood, that nonspecific element of defense that in the presence of natural or specific antibodies effects bacteriolysis in certain diseases. In some cases, also, the natural immune bodies have been shown to be deficient. Lyons has recently called attention to low blood volume in chronic disease.[102] There are probably other factors of which, in our limited knowledge, we are still ignorant.

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Surgical Principles in the Treatment of Infection

EVERY SURGEON during the course of his career builds up a fund of experience with infection upon which he may draw as he practices his art. This fund of experience is largely his own, but to some extent it is the experience of his confreres if he works in an institution where the members of the staff see one another's cases or in a group where there is a free exchange of views, or if he attends medical meetings or reads medical books or magazines.

An old adage says that a fool learns only by his own experiences, a wise man by the experience of others also. On the sum total of these experiences a surgeon builds his philosophy, which directs him in the practice of his art. The surgical philosopher must be open-minded, always reading to test his own experience and the declared or reported experience of others against his own philosophy to see whether they prove themselves true or false. Pasteur once said, 'Don't try to prove you are right, try to prove that you are wrong.'[1]

A surgical procedure, with respect to infection, is the application of some physical force to the body designed to aid the body in preventing or overcoming infection. This physical force is usually applied with a knife, but it also includes the use of many other surgical instruments—scissors, forceps, clamps, needles, retractors, plates, screws, ligatures, sutures, and so on, as well as the hands of the surgeon himself. These instruments always do a certain amount of harm. The surgeon must have it constantly in mind that the good that he accomplishes must outweigh, by as wide a margin as possible, the harm that he does.

We have presented in Chapter II the criteria upon which we base our understanding of what constitutes a surgical infection as distinguished from a medical infection, and we have enumerated the different kinds of surgical infection with which a surgeon has to deal. These fall into three main categories: (1) the infection of operative wounds, (2) infections developing from accidental wounds, and (3) infections already established. In the first two, certain surgical principles apply to the prophylaxis of infection. In all three, certain surgical principles apply to active treatment. These principles will be briefly outlined.

I. SURGICAL PRINCIPLES AS APPLIED TO THE PREVENTION OF INFECTION IN CLEAN OPERATIVE WOUNDS

We must assume that into every operative wound a certain number of bacteria fall, that number depending upon the effectiveness of all of the steps in our elaborate operating-room sterile technique. This varies widely in different operating rooms, and the surgeon must know the conditions under which he is doing his work and reduce to the irreducible minimum the sources of contamination, discussed more fully elsewhere. He must also minimize the local and general conditions favorable to the growth of these organisms, including (A) tissue trauma, (B) hemorrhage, (C) foreign bodies, (D) tissue tension, (E) movement of the part, and (F) secondary contamination. These will be elaborated in order.

A. Tissue Trauma

Every cell in the body has some measure of defense against infection, but it must be a normal, living, fully functioning cell in order to protect itself. A knife cuts across tissue and kills a certain number of the cells in its pathway, but a sharp knife gently applied does less harm than any other instrument. Sometimes the handle of a knife is able to separate tissue layers, thus avoiding the cutting of blood vessels; in certain operations, such as hernia, this is clearly indicated in certain steps of the procedure.

When blood vessels are encountered, they may often be clamped before they are cut, thus making possible the clamping of the vessel by itself without a large mass of the surrounding tissue. If a blood vessel is cut, not only is there loss of blood but also the spot of bleeding masks the bleeding vessel, and not infrequently several attempts at clamping are made before the bleeding is controlled. This damages tissue, and whatever is finally seized by the clamp is cut off from its blood supply by the ligature that has to be put on above the clamp Hemostatic clamps should have small tips, and a minimum amount of tissue about the bleeding vessel should be clamped.

Retraction is necessary to give adequate exposure, but retractors may cause a considerable amount of tissue trauma if improperly placed, too frequently changed, or too strongly pulled upon. Retractors must of course deprive the retracted tissues of their blood supply for the time being; therefore they must be released from time to time. This applies particularly to the retraction of nerves and blood vessels, too much or too prolonged pressure on the former sometimes causing paralysis or paresthesias and, on the latter, thrombosis and embolism. Self-retaining retractors have their place in thyroid or pelvic operations, but they must

be gently applied, and released before the pressure at the site permanently damages the cells.

Prolonged or too forceful clamping of viscera during an operation such as a gastroenterostomy should be avoided if it does more than temporarily retain the contents of the stomach or intestine or temporarily cut off the blood supply at the cut margin of the stomach or intestine.

In the ligation of large blood vessels in continuity, the approximation of the walls should avoid the degree of pressure that injures the intima and produces an intravascular clot. Similarly, when a large vessel is to be cut, it is often wise to tie one ligature near the cut end firmly and a second ligature just proximal to the cut more gently to avoid a similar injury to the intima. Heavy clamps with teeth applied to friable tissues damage them severely, and even when applied to toughened structures like fascia may cause subsequent necrosis. All of these instruments have to be used at times. The surgeon must have it constantly in mind not to do more harm than good with them, for traumatized tissue is a favorable medium for the growth of bacteria.

B. HEMORRHAGE

With reference to his monograph on goitre, Halsted[2] once said, 'The story of goitre is the story of hemorrhage.' That was spoken in the days when we did not know how to quiet down the activity of the toxic gland or reduce the violence of its surging blood supply. Still, thyroid surgery as practiced by some operators is a bloody procedure, with marked loss of red cells at the time of operation and with postoperative extravasation of blood into the tissue. 'Every blood vessel that is clamped, should be tied,' is a good rule for any operative procedure particularly in regions of great vascularity or where the tissues are loose and exert no pressure to stop bleeding, or in cases in which the blood-clotting elements are below normal, as in jaundice or purpura.

Not infrequently during the initial stages of the operation, the blood pressure drops to a point at which many cut vessels do not bleed. As the blood pressure rises, these vessels may open up and pour blood into the tissues. The surgeon must be sure before closure of the wound at the end of the operation, with the state of the blood pressure fully appreciated, that there is no bleeding from vessels that can be tied and no general ooze that can be prevented. If haste requires a compromise with hemostasis, a soft rubber drain should be inserted at the most dependent part of the wound to lead off the blood that may ooze out after closure of the wound. Such a drain should be removed after 24 hours unless there is an indication that the bleeding is continuing. In that case the drain may be left for another 24 hours. If at any time after closure of the wound there is a degree of hemorrhage that the drain cannot lead

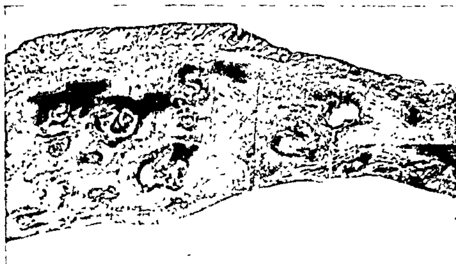


FIG. 1. A comparison of the reaction of the tissues to silk and to catgut, 6th day. Note the relative thickness of the wall and the cellular reaction around the catgut. (From an unpublished monograph by Dr. Peter Vivier)



FIG 2. A comparison of the reaction of the tissues to silk and to catgut 10th day. (From an unpublished monograph by Dr. Peter Vivier)

away, the blood should be aspirated or else the wound should be opened, the clot removed, and the bleeding vessels ligated, because blood in a wound offers a favorable medium for the growth of bacteria and considerably delays wound healing.

C. FOREIGN BODIES

In almost every operative wound, blood vessels require ligation and tissue layers usually require approximation by means of sutures. Ligatures and sutures are foreign bodies that may be relatively inert with respect

to the tissues, for example, silk, cotton, or stainless steel wire; or they may—as does catgut—cause a reaction in the tissues, resulting in their digestion and absorption. A comparison of the reaction of the tissues to silk and catgut is clearly shown in Figures 1 and 2. Chromicized catgut causes a greater reaction than plain gut, but its tanning prevents as rapid digestion and absorption. Such a reaction of the tissues calls fluid and phagocytic cells to the site, and until the gut is dissolved and absorbed there is a little area favorable to the growth of any of the contaminating organisms. This undoubtedly explains the consistently higher incidence of infections in operations in which catgut is used than in cases in which silk is used.[3, 4] (See Chapter VI.) *In clean cases with a minimum of contamination, silk or cotton is the material of choice.* In contaminated or infected cases, however, catgut should be used, because when silk or cotton cases become infected, the nonabsorbable material causes a persistence of the infection until the suture or ligature becomes separated from the surrounding tissue and extrudes itself from the wound. This procedure may take a long time, and in some instances it is necessary to open the wound and remove the stitches in order that the wound may heal. In operating rooms where the contamination is not reduced to a minimum by all the measures that pertain to good sterile technic, or where the surgeons do not *attempt to reduce tissue trauma to a minimum,* or where they fail to mask nose and mouth adequately, silk or cotton should not be used.

Occasionally, a small sponge or a surgical instrument is inadvertently left in the wound. Its presence is usually made manifest in a few days by pain, swelling, or suppuration, which persists until the foreign body is removed. Occasionally a rubber drain or gauze or silk packing is left in the wound and gives rise to the same symptoms. All drains and packing should be marked with a safety pin or otherwise tagged with a thread to prevent such an occurrence.

D. TISSUE TENSION

In order to close a wound there is always a certain degree of pull on the tissues. When a wound is sutured in layers, the tension is exerted not only on the margins that are approximated, but also on the whole sheet of tissue back of the margin. This tension is distributed fairly evenly back of the margin, but the blood vessels contained in the layer are stretched, their lumens narrowed, and the blood supply reduced (Fig. 3). In the exact plane of the suture, whether it is one taken in the margin of a single tissue layer or a massive retention stitch, there is pressure that is maximum on the tissues next to the suture and *within the circle of the stitch.* There is tension on the tissues outside the circle. The pressure kills cells

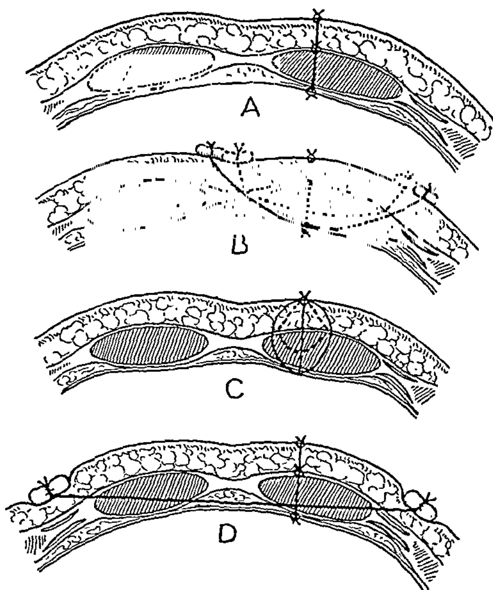


FIG. 4 Diagram to illustrate the rapid cutting of soft tissues and the slow cutting of dense tissues by tight sutures A Effective layer sutures. B. Ineffective 'bolt' sutures. C. Ineffective retention suture D. Effective 'bolt' suture.

E. MOVEMENT OF THE PART

When a wound is sutured, the surfaces are generally held in contact with one another, and though there may be focal necroses at the suture points, the neighboring surfaces are given an opportunity to heal, first by the clotting of blood and plasma with a fibrin network laid down and then by a growth of fibroblasts producing fibrous connective tissue of steadily increasing strength. In the early stages of this process, any movement of the wound surfaces tends to pull them apart and causes

bleeding that separates them still further. The young and delicate fibrous strands may also be torn, up to the time when they have become as strong as the surrounding tissues. Any such death of tissue or collection of fluid between wound surfaces favors infection, because a suitable medium is provided for the growth of bacteria. If wounds are badly sutured or if wounds are left open in such a way as to permit rubbing of wound surfaces, infection by the contaminating bacteria is also favored. This may be an unfavorable feature of too early ambulation.

F. SECONDARY CONTAMINATION

If operative wounds are closed without drainage, there is little likelihood of secondary contamination. True, when the skin stitches are removed, surface organisms may be carried back through the tract of the stitch and become active there, but such infections are not often serious.

If a wound is drained, there is a real opportunity for bacteria from the outside to get in along the drainage tract. This chance is increased as time goes on so that it is advisable not to leave drains in situ after they have done their work, which, in bleeding or oozing, takes 24 to 48 hours. If a deep drain is removed and another inserted in its place, it may carry in surface organisms to the depths.

If a wound is left open, there exists a very favorable opportunity for secondary contamination from the air or from the unmasked nose and mouth, or from the hands of the attendants. Such contamination is to be scrupulously avoided by the rigid procedures of dressing technic outlined by Miles[5] and Williams.[6] Many different kinds of organisms can get a foothold on a freshly cut surface, but as granulation tissue grows, a defense is built up that resists the penetration of most of the air contaminants. However, even with a thick layer of granulation tissue, certain organisms like the hemolytic streptococcus or *Pseudomonas pyocyanea* frequently gain a foothold and invade the tissues. Occasionally they may produce a septicemia and death. Secondary contamination must be reduced to a minimum if secondary infection is to be avoided.[7, 8]

II. INFECTIONS DEVELOPING FROM ACCIDENTAL WOUNDS

A study of civilian accidental wounds is outlined in Chapter VII. The principles involved may be summed up in a few words—'get rid of the contaminating organisms and render the local conditions unfavorable for their growth.'

A. GETTING RID OF THE CONTAMINATING BACTERIA

The organisms that contaminate an accidental wound or a war wound come from the surfaces or pores of the skin, from the object or missile

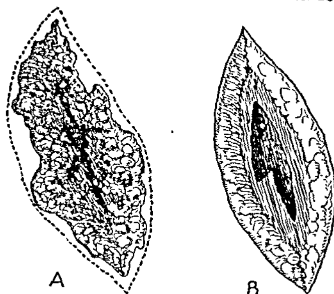


FIG. 5 Diagram to illustrate the removal by the surgical procedure called 'débridement' of dead and injured tissue and the gross contamination of a wound.

causing the injury, or from the dirt and other foreign bodies carried in. They probably number hundreds or thousands and are concentrated here and there around the foreign bodies. Most of them have not been actively growing recently in a human environment, and many of them are in a spore or resting state. If such a wound is washed or the surfaces are rubbed in an effort to get rid of these organisms, they are scattered throughout the wound into all of the interstices and it becomes difficult indeed to remove them. Such wounds should have a *dry* débridement. With the wound covered, the surrounding skin should be cleaned and prepared as for a clean surgical procedure. Then the wound should be débrided, with all of the dead or injured tissue cut away until normal tissues are reached. The margin of skin removal may be narrow, the margin of muscle wide, because muscle is more easily injured. Important nerves and blood vessels should be preserved unless they are injured beyond restoration. All loose bone fragments should come out and of course all foreign bodies must be removed (Fig. 5). In a very dirty wound, one set of instruments should be used for the superficial or dirtiest tissues, and a separate set for the deepest layers. At the end, the wound should be lined everywhere with normal-looking tissues. Then with the great majority of the organisms out of the way, the wound should be flushed for a few minutes with copious amounts of saline. This distributes throughout the wound the organisms that are not washed away, but the numbers are infinitesimal compared with those distributed if the wound is washed before the débridement. Washing has the advantage of

removing small particles of loose tissue or blood that would offer a favorable culture medium for bacterial growth.

All the débrided tissue should be kept together and sent to the bacteriological laboratory for a rapid bacterial analysis. It is not necessary to identify completely and classify all the organisms found, but it is important to report to the surgeon as soon as possible the presence of the hemolytic streptococcus, the *Staphylococcus aureus*, any Gram-negative rods that might inactivate penicillin, and the clostridia of gas gangrene and tetanus. Many of the species present in the débrided tissue will not be represented in sufficient numbers among the organisms that are left to gain a foothold and cause infection, but if the surgeon knows what organisms were there originally, he will be forewarned of the potentialities of infection and be on the lookout for the first clinical signs of their activity.

B. RENDERING THE LOCAL CONDITIONS UNFAVORABLE FOR THE GROWTH OF THE RESIDUAL BACTERIA

With the dirty wound made comparatively clean by the method of débridement and washing described above, the surgeon must observe the same precautions as in a clean operation to minimize those factors that favor bacterial growth, namely, tissue trauma, hemorrhage, tension or pressure, movement of the wound surfaces, foreign bodies, and secondary contamination. Even in the best of hands the infection rate will be four or five times that which develops in clean operative wounds, so that it is advisable to use catgut as suture and ligature material rather than silk even though catgut is undoubtedly more irritating. This is a debatable point, and in certain circumstances, such as tendon suture, the advantages of silk outweigh its disadvantages.

It has been amply demonstrated that the local use of sulfonamides in an effort to minimize the activity of the residual organisms is not advantageous and may even do harm.[9] Apparently there are enough inhibitors present in such a wound to prevent their bacteriostatic action, and they may act as foreign bodies, interfering with wound healing. The local use of penicillin or bacitracin, either in solution or ointment form, is more rational and may be helpful. They are not inhibited by the injured tissue, plasma, or blood, but penicillin may be inhibited by the contaminating organisms present, particularly the Gram-negative aerobic rods frequently there. Although penicillin is of very great importance in controlling infections caused by pure cultures of hemolytic *Staphylococcus aureus* and the hemolytic streptococcus, it is often disappointing and ineffective in the presence of bacterial mixtures.[10, 11] It is obvious that any necrotic tissue left in a wound will prevent contact of any locally applied medicament

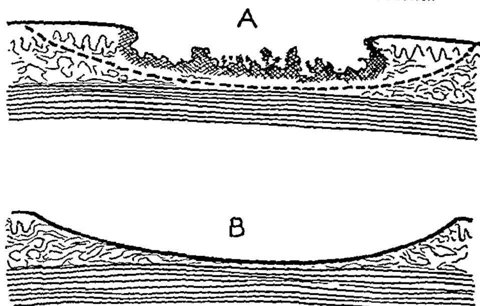


FIG 6. Diagram to illustrate the interference of necrotic tissue with the local application of medicaments.

and render it less effective (see Fig. 6). This has been stressed by Howes, who favors a combination of streptomycin and sulfamylon after proper debridement.[12]

III. INFECTIONS ALREADY ESTABLISHED

When a patient with an established infection comes to a surgeon, the latter is faced with the problem of getting the infection under control. He must be sure that what he attempts in the way of treatment does not make it worse and that the proper treatment is started as soon as possible. The case may be acute, subacute, or chronic. It is important for the surgeon to know the course of the infection up to the time the case was presented to him and the treatment it has already received. His success in controlling the infection depends upon: (A) his knowing what the causative organism or organisms are, (B) the selection of the proper method of treatment, and (C) the proper timing for the administration of that treatment.

A. KNOWING THE CAUSATIVE ORGANISM

An experienced surgeon can frequently make a fairly accurate guess regarding the organism causing an acute infection, because certain species commonly cause the great majority of the cases with which he has to deal, for example, furuncles, carbuncles, suppurative parotitis, and acute osteomyelitis (except in infants) are usually caused by *Staphylo-*

occus aureus. Cervical adenitis from a sore throat and suppurative enosynovitis are usually due to a hemolytic streptococcus, and so on. n many cases, however, it is impossible to tell the organism, and its ature should be determined by culture as soon as possible. At times, it not possible to obtain cultures (if the infection is deep-seated) until a urgical procedure has been carried out. Sometimes, in very sick patients is possible to determine the organism by a blood culture. If a culture annot be taken, the surgeon is handicapped and he must make the best uess his experience will permit.

In chronic cases, very few if any surgeons can guess what the causative rganism is, and certainly no one can tell what secondary contamination present and whether such organisms are merely fellow travelers or are laying a synergistic role in the infection. In such cases, very careful erobic and anaerobic cultures should be made, the susceptibility of the rganisms for the sulfonamides and for the available antibiotics disovered, and the inhibiting action on penicillin determined Blood counts or both white and red cells should be made, and in debilitated individuals t is advisable to test the level of the serum protein and the blood volume.

B. THE SELECTION OF THE PROPER METHOD OF TREATMENT

After any organisms present have been identified, it then becomes ossible to decide intelligently what methods to use for the control of the nfection. With regard to medicinal aids, the one best suited to the infecion should be used. In many cases of mixed infection the treatment hould be combined with two or three of the agents that are effective gainst the causative organisms, sulfonamide, other chemicals, antibiotics, erums, etc. This is discussed more at length in Chapters XIX and XXII With regard to the surgical procedure, the nature of the infection will letermine whether excision, incision, fixation of the neighboring joints, or other specific measures are indicated.

C. THE PROPER TIMING FOR THE TREATMENT OF SURGICAL INFECTIONS

In the early stages of all surgical infections, the reaction of the body s at the portal of entry. Occasionally in children there is no reaction at he portal, the first manifestation of the infection being in some internal issue such as bone. Surgical infections, being local, start usually as a pot or small area of cellulitis. Then, depending on the causative organism and the rapidity with which it can break down tissue, an area of necrosis develops or a localized exudation of pus accumulates.

If the surgeon has decided on medical treatment, it should be given immediately. The timing for surgical treatment depends upon the experience and judgment of the surgeon. In some cases, it is possible and

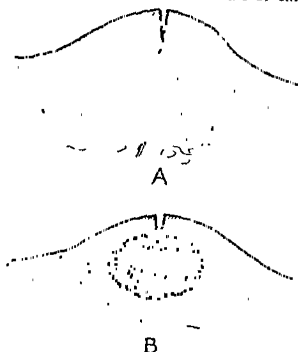


FIG. 7. Diagram to illustrate the unsatisfactory drainage of a cellulitis compared with the satisfactory drainage of an abscess cavity. Surgery must often be delayed until localization has taken place. This affords an opportunity for instituting drug therapy in the stage of cellulitis before there is a breakdown of tissue.

advisable to extirpate completely the infected lesion, as in an acute *appendicitis*, a *gangrenous gall bladder*, or a *suppurative nephritis*. If the body can spare the infected organ, that is the method of choice, and one that avoids disfigurement. At other times merely an incision should be made. In most cases it is advisable to wait until there has been a good deal of central liquefaction as indicated by the sign of fluctuation (see Fig. 7). Early incision into a cellulitis releases tension, but it does not liberate any considerable amount of dead tissue or living organisms or their poisons. Early incision may be necessary, however, in such conditions as Ludwig's angina, where closure of the glottis is threatened, or in hemolytic streptococcus gangrene that is rapidly spreading up the subcutaneous tissues.[13] In such a situation the experience of the surgeon is of very great importance.

In some cases, aspiration is indicated, both to find out the causative organism and to institute the proper local treatment. This can be done satisfactorily only when there is an accumulation of fluid, as in a knee joint or chest. An immediate smear and staining of the exudate may reveal the causative organism and indicate the best treatment, but this should of course be confirmed by culture, because it is not always possible to be sure of a morphological diagnosis. The treatment may be *simple* aspira-

tion and injection of medication at first, and then, if there is no response, a surgical incision must be made *at the proper time.*

If local medication is to be used, the surgeon must remember that contact has to be obtained between the antibacterial agent and the organism. If necrotic tissue interferes with this, the tissue must be surgically removed, but if in doubt, the surgeon may be justified in awaiting a conservative trial of local medication for a few days—if the condition of the patient permits. In this situation also, the proper timing of the operative procedure is of the utmost importance.

D. DRAINAGE

Drains are used in the surgical treatment of infections for two purposes: (1) prophylactic and (2) therapeutic. If a wound is known or believed to be contaminated by pathogenic organisms, a drain may be inserted either to lead off any fluid that may accumulate, such as blood or bile, which may serve as a medium for bacterial growth, or to release a purulent exudate that may be poured out into the wound from the contaminated surfaces during the development of infection.

Such a situation is exemplified by a cholecystectomy Certain cases of acute or chronic cholecystitis are due to bacteria of intestinal origin, and the area of operation during the procedure is not infrequently contaminated with these organisms. Blood from the gall bladder bed or bile from the liver or cystic duct often leaks into the field. A drain judiciously placed into Morrison's pouch will permit these fluids to escape and thus minimize the likelihood of infection. In a study of the development of infection in clean operative wounds it was found that infection developed in a higher proportion of the drained than the undrained cases, but this is surely due to the fact that drains are more often used in those conditions that favor the development of infection.

With an established infection in which an abscess has formed and in which there is a collection of purulent exudate surrounded by a wall of necrotic tissue, drainage is essential not so much for the removal of the exudate, which can be largely evacuated by suction at the time of incision, but for the subsequent elimination of the necrotic tissue in the wall, which gradually liquefies and separates from the living tissue surrounding it.

It is recognized that drains keep wound surfaces apart and to that extent delay the healing of the wound. For that reason they should not be left in after they have served their purpose. The ideal drain is one that will do the most good and the least harm. It should be made of nonirritating material and extend from the center of the contaminated area to the outside by the shortest possible route. The use of drains requires a considerable degree of judgment and experience. There is often a

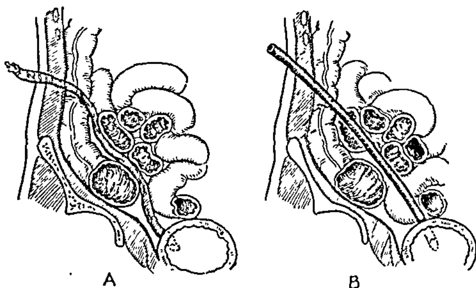


FIG. 8. Diagram to illustrate drainage of the peritoneal cavity by means of soft flexible cigarette drains and by relatively firm tube drains.

difference of opinion among surgeons regarding the use of drains in any given situation. Drainage for bleeding that cannot be completely controlled by the ligature of the blood vessels can frequently be terminated in 24 or 48 hours by withdrawal of the drains. If drains are placed for the possible development of infection, they should be left for at least five or six days, and the day of removal will depend upon the evidence of infection as indicated by the type of draining exudate and the bacterial cultures.

In established infections, drains must frequently be left in place until all the slough has been removed and the drainage tract has become well established. When the drainage is mucoid in character, one can be quite certain that all the slough has separated, but the nature of the infecting organisms as revealed by bacterial cultures or bacterial counts must be considered. When the drains have been placed in a well-established abscess within the peritoneal cavity, they should be left in place for ten days to two weeks in order that the drainage tract may become stiff enough to prevent the retention of a deep pocket. Drainage of the peritoneal cavity is illustrated in Figure 8.

The nature of the drain is not of primary importance, and the same result may be obtained from a soft, collapsible rubber tube, with or without a central wick, or from a soft, non-collapsible, rubber tube or from a China silk tampon packed lightly with gauze. A hard rubber tube or glass rod may cause pressure necrosis on a loop of intestine with which it may be in contact, therefore such drains should be avoided.

The local or systemic use of relatively non-irritating antibacterial

agents such as the sulfonamides and the antibiotics may lessen the necessity for the drainage of contaminated wounds and may shorten the period of drainage of established infections by halting the destructive action of the infecting organisms and favoring the protective mechanisms of the body both local and general.

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CHAPTER XIX

Chemotherapy in Surgical Infections

In reality, chemotherapy antedates medical history. We know from a study of the old Egyptian papyri that infections similar to those we see today occurred following accidents and war injuries in the days long before these records were made.[1] Chemicals were used in their treatment, for on record are the very prescriptions possibly handed down through many generations of Egyptian doctors. Similarly, all through the medical literature both before and after Hippocrates are found the records of the use of chemicals both internally and externally in the treatment of infections.

It was logical, therefore, that when specific organisms were found to be responsible for these infections, numerous attempts should have been made to combat these organisms by means of chemicals. A promising lead was opened up when Weigert[2] found that certain stains or dyes were taken up by organisms, thus making them clearly visible under the microscope.

Koch,[3] Von Behring,[4] and others had been unsuccessful in their attempts to find chemicals effective as internal disinfectants against bacterial diseases. They had come to the conclusion that a successful search would be impossible, because they believed that the organs and tissues of the body would be more susceptible to the disinfectants than the organisms themselves and that any internal administration of such chemicals would kill the host before it reached the offending organisms. However, Ehrlich[5] vigorously pursued his search for chemicals that would have a selective action on bacteria and that would be relatively nontoxic to the host. In this search, he was ably supported by Hata[6] and Browning.[7] It is of interest that we are indebted chiefly to a German and a Japanese for the basis of our chemotherapy, and that Ehrlich said at the International Congress of Medicine in 1913, 'In the world of science all national barriers have disappeared.'[5]



FIG. 1. EHRLICH. Dr. Paul Ehrlich, the 'Father of Chemotherapy,' in 1915 predicted a wide expanse of this field. He was a scientific internationalist in the truest sense of the word. (Courtesy of New York Academy of Medicine)

1. THE DYES

The lead offered by the dyes was eagerly pursued by many workers in several countries, because it was found that dyes had a selective action on bacteria, potent against some and inert against others. Furthermore, multitudinous variations could be made by introducing or withdrawing radicals of different kinds that might add to their potency against various species of bacteria or detract from their toxic effects on the tissues of the host. One group of dyes seemed to be particularly promising, namely the acridines. Ehrlich[8] demonstrated that one of these, acriflavine, had



FIG. 2. HATA. Dr. Sahaohiro Hata, an associate of Ehrlich, believed that chemotherapeutic agents could not be successful unless they could act in the presence of body fluids.

a powerful lethal action on trypanosomes, and Neufeld [9,10] found it to be effective against the pneumococcus and the chicken cholera bacillus.

Browning [11,12] in England became one of the chief advocates of dye therapy particularly for local application in wound infections or for prophylaxis against infection in accidental wounds and war casualties, but Fleming [13] warned against their danger, for he had demonstrated their deleterious action on leukocytes and tissue cells.

From these studies gradually emerged three dyes that were given extensive clinical trial both with systemic administration and local application particularly in the treatment of hemolytic streptococcus and *Staphylococcus aureus* infections. These were acriflavine, gentian violet, and mercurochrome; and hundreds of cases were treated by one or an-



FIG. 3. DOMAGK. Dr. Gerhard Domagk aroused renewed interest and broadened the field of chemotherapy by the discovery of the antibacterial action of the sulfonamides. (Courtesy of New York Academy of Medicine)

other of these dyes. Many favorable reports appeared in the surgical literature,[14-16] but gradually evidence began to accumulate that these dyes were not only ineffective in most cases but positively harmful. Finally deaths were attributed to them, and they gradually fell into disrepute.[17]

II. THE SULFONAMIDES

A decade of reaction followed these reports, and the surgical profession went into a state of antiseptic nihilism, so that as late as 1936 at a meeting of the American College of Surgeons, the consensus of opinion was that chemical agents were of no avail in the prophylaxis or treatment of surgical infections either by general or local application.[18] At that very meeting, however, someone had the temerity to suggest that some en-



FIG 4. COLEBROOK Sir Leonard Colebrook was the first to demonstrate the life-saving action of sulfanilamide in the treatment of human hemolytic streptococcal infecti and specifically puerperal fever.

couragement was to be found in a new dye of the azo series call prontosil recently advocated by Domagk[19] and by Colebrook.[20]

All during this decade of reaction, the search for a more effective ai less harmful dye continued in a few laboratories. Finally persistence w rewarded, and Domagk[19] presented his experiences with prontosil, whi when given to mice by mouth he had found effective against many leth doses of hemolytic streptococci injected into the peritoneal cavity of tl mice. Domagk's results were confirmed by Levaditi and Vaisman[21] ar by Nitti and Bovet[22] and put to early clinical trial by Colebrook an Kenny[20] who had the rare opportunity of treating a large number c cases of puerperal fever (caused by the hemolytic streptococcus) : Queen Charlotte's hospital, which was designated to receive cases of th: disease from all parts of London. As his experience accumulated, it wa clearly demonstrated that he was able, with the use of this new dye, no only to lower mortality from that disease but also to minimize the intra peritoneal spread of the infection and shorten the period of illness ii

the cases that survived.[23] In the meanwhile it had been demonstrated by several French observers [24] that the active agent of the dye was in the colorless fraction known as sulfanilamide, and Colebrook confirmed the clinical effectiveness of this simpler compound.[25]

Colebrook's clinical results were supported by laboratory studies in which he showed that the blood of man or animals treated with sulfanilamide was more *bacteriostatic* than the blood of normal individuals, but he found that it was not *bactericidal* except for very small inocula. This was also true when the drug was added to blood in vitro. Prontosil had the same effect in vivo but not in vitro. Colebrook rightly concluded from these observations that the prontosil broke down to the active sulfanilamide, and the role of the drug in therapeusis was to interfere with the multiplication of the organisms within the body.

During the next two or three years, the use of sulfanilamide, which was cheap and readily obtainable, became first widespread and then promiscuous. It was used by everyone for all types of infections and for many cases of illness before even the diagnosis of infection had been made or the causative organism found. Only in those institutions where it was possible to determine the nature of the etiological agent could anything be learned regarding the limitations of the sulfonamides in surgical infections.

A. THE MODE OF ACTION OF THE SULFONAMIDES

1. *Bacteriostasis*

As has been stated above, Colebrook [23] demonstrated that sulfanilamide when administered to patients acutely ill with hemolytic streptococcal infections enhanced the bacteriostatic action of the patient's blood when then tested in vitro.

The precise methods worked out by Marshall and his co-workers [26] for demonstrating the concentration of sulfanilamide in the blood in milligrams per hundred cubic centimeters made it possible to show that this bacteriostatic action varied with route and amount of drug administration. After a single dose by mouth, the drug level reached a peak in about two hours, and with normal kidney function, it practically disappeared in four. If given intravenously, depending on the *speed of administration*, it reached a peak at the end of the treatment and then rapidly declined. If applied to the peritoneal cavity or a wound, the drug was more slowly absorbed. Colebrook's observation that sulfanilamide was bacteriostatic rather than bactericidal, except for very small inocula of organisms, has been confirmed repeatedly by many other workers in this field.

2. Triangular Interaction

How a bacteriostatic agent in the blood stream can check the invasive properties of certain organisms like the hemolytic streptococcus has been the object of extensive researches. Any human infection may be considered a battle between the organism and the body. The various factors entering into the struggle on the two sides have been dealt with to some degree in earlier chapters. The problem has been studied extensively by Menkin [27] and Duran-Reynals.[28]

When drugs are given during the course of an infection, a third factor comes into play, and it may affect the outcome of the battle by reacting either with the organisms or with the host. Thus there is a triangular interaction—bacteria with drug, bacteria with host, and drug with host. A drug may interfere with bacterial growth either directly or indirectly.[29] Woods's [30] demonstration of the inhibiting action of para-aminobenzoic acid served as a basis for a theory of direct action, namely, that bacteria that ordinarily utilize this chemical for their growth will, if it is not available, take up instead the related substance, sulfanilamide, if that is present. Then, having ingested it, they find that it does not satisfy their metabolic requirements, so that they cannot multiply or produce their poisons. If para-aminobenzoic acid is present in sufficient quantity, however, the organisms utilize it and multiply. Thus, although sulfanilamide may be capable of preventing the growth of bacteria in the blood stream, or in recently developing metastatic foci, it may be ineffective in an area of necrotic tissue containing para-aminobenzoic acid. It is well known that the organisms may be held in check and that all clinical evidence of infection may temporarily disappear during drug administration, but after treatment has stopped, the bacteria become active again, suggesting that there has been a localized abscess with necrosis of tissue isolated from both the drug and the defensive forces of the body. When such foci become active, the whole process of tissue destruction and bacterial dissemination may recur with bacterial progeny more resistant than before to the inhibiting action of the drug.[31]

The foregoing observations must be kept in mind by the surgeon who would utilize the sulfonamide drugs most effectively. They are only *adjuncts to surgery in surgical infections* and cannot be expected to be successful without a surgical procedure if there has been extensive tissue breakdown.[32]

3. Pharmacology and Toxicology

The relation between drug and host constitutes the pharmacology and toxicology of the drugs. Some variations with respect to the different members of this group should be kept in mind. The sulfonamides are all

rapidly absorbed from the alimentary tract and permeate uniformly all the organs and tissues of the body.[33] For some reason, sulfathiazole reaches a concentration in the spinal fluid less than half that it reaches in the blood, whereas sulfanilamide and sulfadiazine reach an almost equal level.[34] Sulfathiazole is the member most rapidly eliminated, and it is therefore more difficult to maintain at a constant level in the blood. Sulfadiazine is the one most stable in its blood level. Absorption from wound surfaces is in proportion to the solubility of the drug. Sulfanilamide, being soluble in about 1 per cent, is very rapidly absorbed, whereas sulfathiazole and sulfadiazine disappear much more slowly. The latter may last for three or four weeks if deposited in a wound. The sodium salts of sulfathiazole and sulfadiazine are very soluble and are readily absorbed from wound surfaces, although their alkalinity seems to have a deleterious effect on wound healing.

A certain portion of each of the drugs is inactivated in the body by the process of acetylation that takes place in the liver.[35] Fatalities have resulted from the use of sulfapyridine, sulfathiazole, and sulfadiazine, but not from sulfanilamide, because of crystallization or precipitation in the tubules of the kidney or in the ureters. This danger has been minimized by the knowledge that crystallization occurs most often when the urine is acid and concentrated. It can be obviated in large measure by insuring adequate intake of fluid and by alkalinizing the urine by sufficient doses of sodium bicarbonate.[34] If blockage occurs from crystals, prompt action may be necessary to prevent the development of uremia. Action consists in an increase of fluid intake, in alkali administration, and in some cases in mechanical removal of the crystals from the ureters.

Sulfanilamide gives the most diverse toxic effects, and sulfadiazine the least of the whole group.[32] It has been mentioned above that if toxic effects appear early, they depend upon individual idiosyncrasies in patients who have never before come in contact with the drug, but if treatment has been prolonged they depend on sensitivity. Sensitivity for one member of the group may or may not indicate a sensitivity for the others Drug fever is perhaps the most baffling of these manifestations, because it is difficult to know whether the fever indicates a reactivity of the infection or a toxic reaction to the drug. Clinical manifestations of one sort or another are usually evident if it is a reactivity of the infection.

4. *Dosage*

After a considerable period of experimentation, it has been found that 0 1 gm. per kilogram of body weight in 24 hours divided into 6 doses (every 4 hours) will maintain a therapeutic level in the blood in most cases. It is necessary to test the blood level periodically and vary the doses accordingly to maintain the desired concentration. This is generally

5 mgm. per cent for sulfanilamide, and 10 mgm. per cent for the other sulfonamides. For the average patient 5-7 grams may be given as an initial dose and thereafter 1 gram every 4 hours.[37]

B. The Limitations of Sulfonamide Therapy

1. *Bacteriological Limitations*

The most important limitations of sulfonamide therapy are bacteriological. It has been found that infections caused by the hemolytic streptococcus, such as puerperal fever, usually respond to sulfanilamide, whereas infections caused by staphylococci, pneumococci, or nonhemolytic streptococci are frequently resistant. Even such hemolytic streptococcus infections as meningitis, formerly always fatal, yield to the drug, and peritonitis, frequently grave in its outcome, comes under control. Suppurative arthritis, osteomyelitis, and tenosynovitis, all due to hemolytic streptococci, and which used to require surgery for their control (and even then without hope of normal restoration of function), frequently resolve under sulfanilamide therapy if given in the early stages of the disease.

The failure of sulfanilamide in surgical infections other than the hemolytic streptococcal led to the search of other derivatives that might have a wider bacterial range. Sulfapyridine was then found to control the pneumococcus,[38] and sulfathiazole showed its superiority against the gonococci and some of the staphylococci.[39] Furthermore, in experimental infections, the latter was more effective than the others against the gas-gangrene organisms.[40] Sulfadiazine, perhaps as wide in its bacterial coverage as any of the others,[41] and better than they in urinary infections, because of its greater safety was still of doubtful potency against staphylococci and the clostridia. Clinical infections caused by the latter organisms have a resistance to the sulfonamides that is at least partly due to the early and rapid breakdown of tissue mentioned below.

2. *The Time Limitation*

The second most important limitation is that of time. Hemolytic streptococci habitually produce a diffuse inflammatory reaction in the tissues of the host, which is called cellulitis. The suddenness and violence of the symptoms often bring the patient promptly to the doctor in the first few hours of the infection. There are redness, swelling, induration, and tenderness, which may last for several days before there is any breakdown of tissue. Such an inflammation frequently takes place in lymph glands that drain an area infected with this organism. This gives the doctor an opportunity to administer the drugs early in the course of the disease before it has become established in metastatic areas. Infections with the

hemolytic *Staphylococcus aureus* or the anaerobic organisms are characterized by an early breakdown of tissue. This breakdown in itself would make these infections less susceptible to sulfonamide therapy than the more diffuse inflammations caused by hemolytic streptococci. Early administration of these drugs is much more effective than late administration.

3. *The Limitation of Impenetrability*

The third limitation is the impenetrability of the drug into a focus of necrosis because of thrombosed blood vessels in the periphery of the lesion. Whereas sulfanilamide readily enters the blood from the gastrointestinal tract or from the tissues, when introduced into the subcutaneous areas or into a wound, and diffuses out of the blood stream readily into all of the body tissues, it is hindered from entering an infected focus where there has been a breakdown of tissue. Whenever it reaches such a focus, it encounters the fourth limitation mentioned below.

4. *The Limitation of Sulfonamide Inhibitors*

The fourth limitation is the inactivation of the drug in the presence of or by certain protein-split products present in necrotic areas. These have been called 'inhibitors,' and they often completely nullify the bacteriostatic action of the drugs. Lockwood[42] has demonstrated the inhibiting action of peptones, and Woods[30] has shown the same effect with para-aminobenzoic acid, which is present in large quantities in all purulent exudates and necrotic tissue. It is known that this chemical is a readily utilizable nutrient element for bacteria, and that even a small amount will inactivate a large amount of sulfanilamide.

Sulfanilamide, which acts best in an alkaline medium, is also inhibited by many of the acids that are formed in necrotic tissue and exudates.[43]

5. *The Limitation of Toxicity*

The fifth limitation of sulfanilamide success in the treatment of infections is its toxicity. This toxicity is varied in its manifestations but is usually not serious, except that treatment generally has to be discontinued when signs of it appear. These have taken the form of nausea, cyanosis, fever, rash, anemia, leukopenia, jaundice, hepatitis, and psychic disturbances. Occasionally, the symptoms appear promptly after the first administration of the drug, in which case they indicate some idiosyncrasy; but usually they develop after prolonged use of the drugs, at any time after the first week. They do not run parallel with high levels of the drug in the blood and indicate some unknown changes taking place in certain organs or tissues of the body without our knowledge of the mechanism thereof.[44]

6. *The Limitation of Drug 'Fastness'*

The sixth limitation to the successful use of sulfanilamide lies in the fact that certain organisms can build up a fastness to the drug. This may simply represent a survival of the fittest and an inheritance of resistant characteristics in the progeny of the surviving organisms, or a real adaptation on the part of the organisms to the drug environment. In any event, during the course of treatment, particularly if small doses are used, there may be a return of symptoms of the infection and cultures may show a resistance to the drug in vitro that the original cultures did not have.[43] Thus it is conceivable that in any community there may be built up many resistant strains of hemolytic streptococci able to produce disease even under sulfonamide therapy in the same way organisms could before the advent of these drugs. With any bacteriocidal agent, its effectiveness, both in vitro and in vivo, against any given organism depends in large measure on the number of organisms with which it comes in contact. The organisms will vary in their susceptibility according to their stage of development as well as their natural resistance. A certain percentage will succumb promptly, whereas others will yield slowly or not at all.

All of the limitations mentioned above indicate (1) the importance of a bacteriological analysis for all infections, (2) a test for the susceptibility of the organisms, and (3) early treatment with doses as large as can be tolerated without toxicity. There should always be keen watchfulness for any indications of toxicity, as well as for unquestionable evidence of therapeutic response in all cases of infection treated with the sulfonamides. Therefore too much must not be claimed for them or expected of them.

C. OTHER DERIVATIVES OF THE SULFONAMIDE GROUP

The limitations mentioned above, which slowly became apparent as experience accumulated with the use of sulfanilamide, clearly pointed to definite indications for its use and the possibility of predicting fairly accurately those cases that would do well and those that would fail with sulfanilamide treatment. The indication par excellence for sulfanilamide is the early hemolytic streptococcal lesion; for pneumococcal infections, sulfathiazole or sulfadiazine; for urinary tract infections, sulfadiazine. For staphylococcal and clostridial infections, sulfathiazole is the best of the sulfonamides, but they frequently fail to yield to this drug, possibly because it fails to reach the infection. On the other hand penicillin has demonstrated its superiority, particularly in pure staphylococcal infections,[43] in the very important field in which the sulfonamides are weak. Although sulfapyridine demonstrated its effectiveness in pneumococcal infections as rapidly as sulfanilamide had with the hemolytic streptococcus, it had two serious disadvantages: its nauseating properties and

Case I: J.M, Age 46. No. 580100

DIAGNOSIS. Suppurative tenosynovitis of the ulnar bursa (due to hemolytic streptococcus), diabetes mellitus, drug fever.

HISTORY. Forty-eight hours before admission, a wire entered the palm of the patient's hand while he was at work plumbing. He covered the wound with adhesive and 6 hours later noted pain and tenderness in the hand. He soaked it in hot epsom salts solution. Shortly afterward he had a shaking chill, with pain and tenderness steadily increasing.

PHYSICAL EXAMINATION. On admission his temperature was 102.4° F. He had pain on attempted flexion of the fingers of the right hand and swelling of

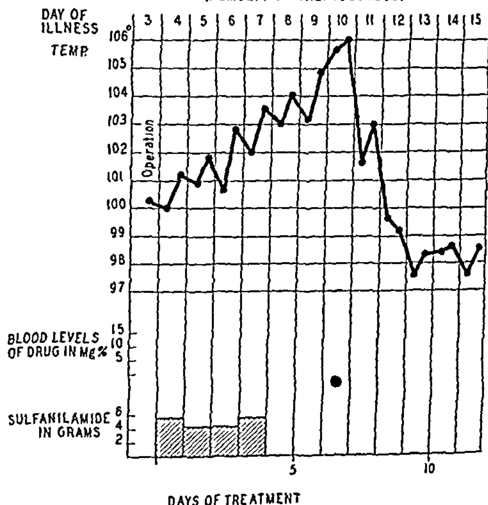


FIG. 5 CASE I. Sulfanilamide helped to control the infection but caused an alarming fever lasting four days after the drug had been stopped (Figs. 5-15 are from Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

the ulnar side of the palm extending up above the wrist, with a small puncture wound just proximal to the transverse palmar crease. At operation it was found that a small tract extended from the puncture wound to the distal margin of the ulnar bursa, which was filled with thin seropurulent exudate. There was no connection between the ulnar bursa and the tendon sheath of the little finger. There was no pus in the mid-palmar space.

OPERATION. Incision was made on the radial margin of the ulnar bursa in the palm and on the ulnar margin of the forearm just above the wrist, thus freely draining the ulnar bursa. Culture revealed a hemolytic streptococcus

COURSE. The patient was given sulfanilamide, 5 4 gm. The local process subsided rapidly but the temperature showed a rise to 104° F. The infection was obviously under control by the fourth day so that the drug was discontinued. Temperature continued to rise for 3 more days and then fell, reaching normal in the next 48 hours. Perfect function of the hand was restored. The sulfanilamide undoubtedly played an important role in the control of the infection but was the cause of the fever, which lasted 3 days after the drug was stopped. The diabetes was controlled with insulin but may have played a part in this unusual reaction (Fig. 5)

This case illustrates the prompt control of an acute tendon-sheath infection by surgery aided by sulfanilamide, but recovery was complicated by drug fever lasting 3 to 4 days after the drug had been discontinued.

Case II L.C. Age 39. No. 563206

DIAGNOSIS. Cholelithiasis and postoperative pneumonia.

HISTORY. Typical symptoms of gall bladder disease with stones; duration, two years.

PHYSICAL EXAMINATION. Moderate obesity. No jaundice, no recent cold. Moderate tenderness in gall bladder region.

OPERATION. Cholecystectomy was done for a chronically inflamed gall bladder filled with stones. The wound was drained.

COURSE The day after operation, the patient's temperature ranged around 101° F. and during the night mounted to 104° F. with typical signs and symptoms of pneumonia appearing on the second day. She was given sulfathiazole, which was continued for 5 days, when signs in the chest had cleared and temperature approximated normal. Sputum cultures for pneumococcus revealed Type VI Hospitalization was not prolonged by the complication. (Fig. 6)

This case illustrated the prompt response of postoperative pneumonia to sulfathiazole.

Case III. J.S. Age 14. No. 654193

DIAGNOSIS. Acute appendicitis with perforation Acute peritonitis.

HISTORY Typical symptoms of acute appendicitis of 19 hours' duration

PHYSICAL EXAMINATION Direct tenderness over entire right abdomen and right flank with direct and indirect rebound tenderness referred to the R.L Q and spastic rectus muscle.

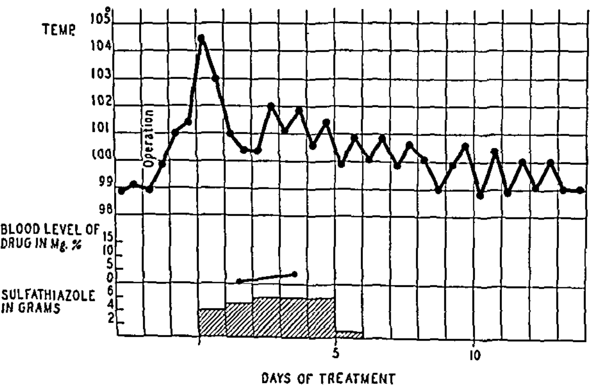


Fig. 6 Case II. The value of the drug in this postoperative pneumonia was fairly clear cut. (Courtesy of Dr. Henry Cooper)

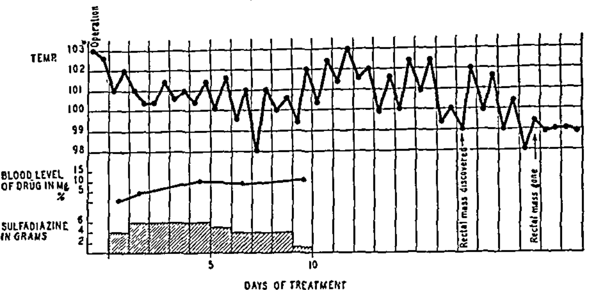


FIG. 7. CASE III. The value of the drug in this case was questionable. (Courtesy of Dr. Grant Sanger)

OPERATION. At operation, a perforation was seen in the middle of the appendix, which had been covered by fresh adhesions. There was a large amount of free, turbid fluid in the neighborhood of the appendix and in the pelvis. The appendix was removed by ligating the base and cutting it off close to the chromic ligature with a knife dipped in carbolic. About 5 gm. of sulfanilamide powder was sprinkled around the neighboring portion of the peritoneal cavity. A drain was placed in the right lumbar gutter and brought out through the center of the wound. Five gm. of sulfanilamide powder was distributed through the muscles and subcutaneous tissue. The skin was left open and tamponed with China silk. Cultures yielded *Clostridium welchii, Escherichia coli, Pseudomonas pyocyanea,* and nonhemolytic streptococcus.

COURSE. She was given sulfadiazine by mouth and her temperature gradually subsided during the course of the next 7 days, and then started to rise without any evidence of reactivity of the infection. It was thought that she had drug fever so the drug was stopped on the eleventh postoperative day. The temperature continued to rise for the next 4 days, during which time a tender mass was felt in the pelvis. No further drug was given, but the temperature gradually subsided and the mass disappeared spontaneously, temperature reaching normal on the twenty-first postoperative day. (Fig. 7.)

This case illustrates the difficulty of determining whether a fever developing during the course of drug therapy is due to the drug or a residual or recurring infection. The benefit from the drug was questionable in this case.

Case IV· E.G. Age 19. No. 627072

DIAGNOSIS. Acute appendicitis with perforation and acute diffuse peritonitis

HISTORY. Forty hours before admission the patient was awakened from sleep by a severe periumbilical pain, which continued with some abatement during the first 24 hours and then shifted to the R L Q. She tried to eat a Thanksgiving dinner with all the trimmings, and 5 hours later had sudden exacerbation of the pain that doubled her up.

PHYSICAL EXAMINATION. Temperature 99.8°. Spasm in the R.L.Q. with direct and rebound tenderness in all areas of the abdomen referred to the R.L.Q. White blood count, 21,500. Polys 84 per cent.

COURSE. At operation, a gangrenous appendix was found with a perforation sealed off by adhesions. Approximately 200 cc. of yellowish white, slightly fecal smelling fluid was aspirated from the free abdominal cavity. The appendix was removed and the stump inverted. Penrose drains were placed down into the region of the appendix and the ileal loops that had been adherent to the appendix. The peritoneum was closed around the drains and the muscles loosely approximated. The skin was left open except for one stitch at each extremity of the wound, the central portion being packed with plain gauze saturated in zinc peroxide suspended in distilled water. Cultures of the peritoneal fluid revealed only nonhemolytic streptococci (probably an anaerobic streptococcus because of the fecal odor which is not usually associated with an aerobic streptococcus). She was given infusions with 2½ gm. of sodium

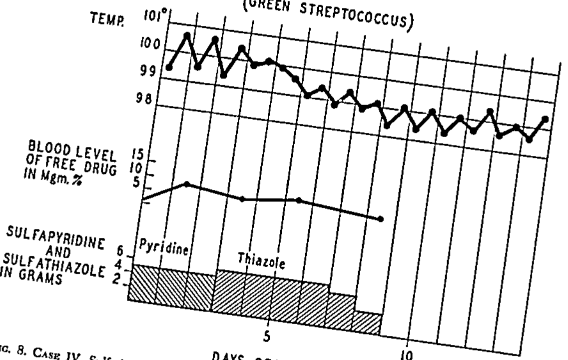


FIG. 8. CASE IV. Sulfathiazole seemed to be effective while sulfapyridine was not. (Courtesy of Dr. Edward Hirsch)

sulfapyridine every 12 hours for 3 days and after that 1 gm. of sulfathiazole by mouth every 4 hours for 6 more days. Her postoperative temperature never went above 100.6° F. The drains were removed one by one until on the seventh day all were out. The wound was then brought together with flamed adhesive. She left the hospital on the fifteenth day, and 17 days later the wound was completely healed. (Fig. 8.)

This case represents a perforated and gangrenous appendicitis with diffuse peritonitis, potentially serious but with a very smooth course following an appendectomy, with the help of sulfapyridine and sulfathiazole. Such a result, however, might have been obtained without the use of the drugs.

Case V: P.W. Age 30. No. 654137

DIAGNOSIS. Hemolytic staphylococcus septicemia with cellulitis of the face.

HISTORY. Two weeks before admission the patient cut his upper lip while shaving. The next day it became swollen, with very rapid extension, fever, and purulent discharge. Three days before admission he went to another hospital where he was treated with sulfathiazole, 2 gm. every 4 hours. Blood culture revealed hemolytic *Staphylococcus aureus*. The left eyelid became

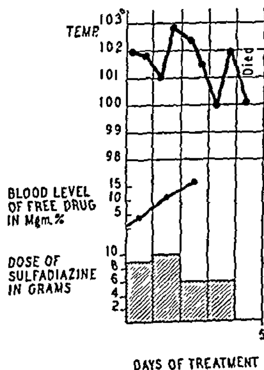


FIG. 9 CASE V. Sulfadiazine had no demonstrable effect in this serious case of staphylococcus septicemia.

greatly edematous, and a diagnosis of cavernous sinus thrombosis was made. The patient was sent, therefore, to the Presbyterian Hospital (New York City) for heparin treatment.

PHYSICAL EXAMINATION. An acutely ill, delirious man with an upper lip markedly swollen and ulcerated. The entire upper face was markedly indurated and bluish red in color, the induration extending downward over the left side of the neck and upward around the left eye and forehead with marked chemosis. The heart was overactive with a loud presystolic rumble and an aortic diastolic murmur (an old history of rheumatic fever). There were numerous small pea-sized hemorrhagic areas of induration over the right abdominal wall.

COURSE. He was immediately started on sulfadiazine with heparin. The blood culture revealed hemolytic *Staphylococcus aureus*. The spinal fluid showed no growth. Sulfadiazine level quickly rose to 22 mgm per cent. Clotting time was kept over one hour. On the day after admission, he was a little more rational, but his right arm began to swell. Other hemorrhagic subcutaneous foci appeared. On the third day the blood culture was still positive. The neck became resistant. Two fingers of the left hand became gangrenous. On the fourth day, hemiparesis developed and he sank into a deep coma with convulsions. He died early on the fifth day. Autopsy revealed endocarditis with multiple abscesses of the brain and abdominal organs. Cavernous sinus thrombosis could not be demonstrated. (Fig. 9.)

This case illustrates the failure of sulfathiazole to control a serious staphylococcal infection of the face, which sulfadiazine and heparin could not halt in its terminal stages.

Case VI. M.R. Age 25. No. 696184

DIAGNOSIS. Septic endometritis due to hemolytic streptococcus.

HISTORY. Eight days before admission there was a rapid onset of generalized headache, sore throat, with swelling of the glands of the neck followed by shaking chills. These symptoms lasted 5 days. They disappeared for 2 days and then the fever, chill, and headache recurred with no sore throat. The patient denied abortion or any other genital tract disturbances.

PHYSICAL EXAMINATION She was acutely ill, with temperature 106°. The neck glands were palpable but not greatly enlarged. There was a loud systolic cardiac murmur. The lower abdominal muscles were slightly spastic. Uterine bleeding was evident with a strong odor to the discharge. The cervical canal admitted one finger and the uterus was slightly enlarged. White blood count revealed 30,000 cells with Polys 89 per cent. Blood culture and vaginal culture yielded a hemolytic streptococcus. Throat culture produced no hemolytic organisms.

COURSE. She was given sodium sulfadiazine intravenously and within 24 hours her temperature reached normal and stayed there. Sulfadiazine was given, 6 gm. a day for 4 days and then 4 gm. a day for 2 days, her blood level reaching 15 mgm per cent on the fourth day. (Fig 10.)

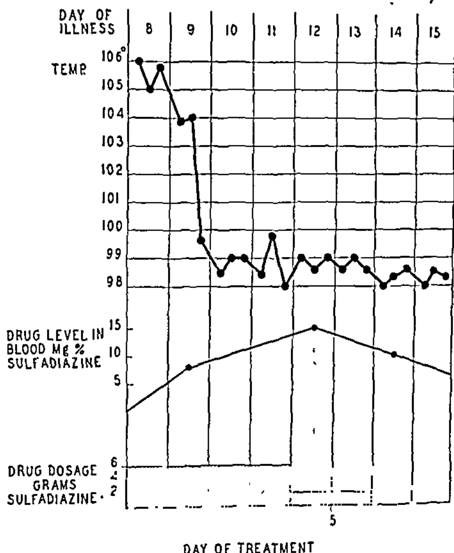


FIG. 10 CASE VI. Sulfadiazine yielded a brilliant result in a serious case of hemolytic streptococcus endometritis and septicemia (Courtesy of Dr. Sidney C. Werner)

This case illustrates the prompt response to sulfadiazine of a hemolytic streptococcus septicemia due to septic endometritis.

Case VII: C.L. Age 27. No. 671296

DIAGNOSIS. Abscess of neck due to an anaerobic nonhemolytic streptococcus

HISTORY. One week before admission, the patient developed a toothache in the left lower jaw. He went to a dentist who extracted the offending molar. The next day there was more swelling of the jaw and trismus. Two days later in the dental clinic, a small incision was made in the mouth lateral to the tooth

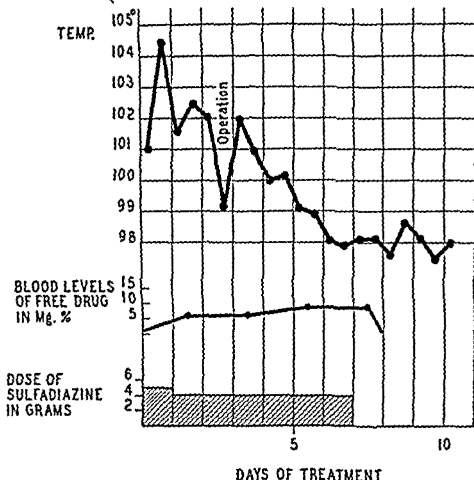


FIG. 11. CASE VII. Sulfadiazine helped to control this anaerobic cellulitis of the neck but did not obviate surgery. (Courtesy of Dr. J Cracovaner)

socket, but the swelling of the jaw and neck continued, and difficulty in swallowing gradually developed.

PHYSICAL EXAMINATION. There was an elevation of the floor of the mouth and the anterior pillar of the fauces was swollen. The buccal mucosa lateral to the left lower molars was indurated. There was marked cellulitis of the left side of the neck extending over to the right submaxillary region.

COURSE. He was given 5 gm of sulfadiazine during the first day. His temperature mounted to 104.4° F. on the afternoon of the day of admission, when an aerobic blood culture was taken, which showed no growth. His temperature fell on the second day to 102° F., and the dose of sulfadiazine was reduced to 4 gm. a day. On the fourth day, the central portion of the

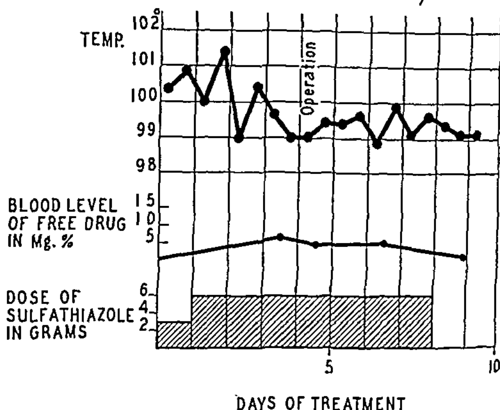


FIG 12. CASE VIII. Sulfathiazole helped to control this infection but did not obviate surgery. (Courtesy of Dr. Edward Hirsch)

swelling softened and a generous incision was made. A large abscess cavity was entered in the submaxillary region and pus was evacuated, yielding on culture an anaerobic nonhemolytic streptococcus. Thereafter, the temperature subsided, on the second postoperative day reaching normal, where it remained until his discharge 5 days later. Sulfadiazine was stopped on the fourth postoperative day. (Fig. 11.)

This case illustrates the partial control of a nonhemolytic anaerobic streptococcus with sulfadiazine and the necessity for the surgical evacuation of the necrotic tissue and purulent exudate.

Case VIII: J.H. Age 15. No. 456067

DIAGNOSIS. Abscess of arm due to streptococcus viridans.

HISTORY. Two weeks before admission while playing football this boy fell and hit his left elbow. One week later the elbow began to swell with pain and tenderness. About the same time he noticed a small area of infection on the back of the third finger.

PHYSICAL EXAMINATION. On the inner side of the extremity extending from the midarm to the upper forearm, there was a large area of cellulitis with edema, hyperemia, and tenderness. The central portion of this area was very firm, with pitting on pressure. On the dorsum of the third finger was a small infected area, which looked like an abrasion rather than a furuncle.

COURSE. He was given 1 gm. of sulfathiazole every 4 hours, with continuous hot dressings and elevation for the arm. The infected area on the dorsum of the finger was found to surround a small sinus opening leading into the subcutaneous tissues. Cultures from the finger yielded a green streptococcus. The day after admission his temperature rose to 101.2° F. but thereafter subsided, reaching normal on the fourth day. During this time the cellulitis subsided, but on the fifth day the central area became fluctuant. This was incised and drained, the culture yielding the same organism that was found in the hand. His temperature remained normal. The sulfathiazole was continued for 8 days and he left the hospital on the tenth day. (Fig. 12.)

This case illustrates the resolution of a peripheral cellulitis following the administration of sulfathiazole with a breakdown of the center of the infection requiring surgical evacuation.

Case IX: J.L. Age 22. No. 630051

DIAGNOSIS. Acute purulent pansinusitis. Hemolytic streptococcus septicemia.

HISTORY The patient had an upper respiratory infection for one week, with headache chiefly around the left eye, ear, and left side of the face. The pain was agonizing for the last two days. He had a shaking chill on the day of admission.

PHYSICAL EXAMINATION. Patient's temperature was 102.2° F. There was a purulent discharge from the nasal sinuses. The left eardrum was markedly retracted and there was acute tenderness over the sinus on the left side. There was moderate swelling of the eyelids with injection of the left sclera.

COURSE. Blood cultures yielded a hemolytic streptococcus. The patient was put on sulfanilamide, 5.4 gm. for 2 days. His temperature moderated slightly but not abruptly. The amount of drug was increased to 7.2 gm. on the fourth day of treatment, and on the fifth day, he was given 3 6 gm. of sulfanilamide and 5 gm. of sulfapyridine, because it was thought that sulfapyridine might be more effective. The blood cultures taken the evening of the day sulfanilamide was begun showed no growth. Cultures taken later, on the fourth and fifth days of treatment, when the temperature reached 104.8° and 104.4° respectively, were also negative. On the sixth day of treatment, he had a chill but did not appear acutely ill. The drug levels gradually mounted from 5 mg. on the first day to 14 mg. on the seventh day of treatment. The red blood count had fallen so that it seemed advisable to stop the drug and he was given two small transfusions. On the day following the stopping of the drug, his temperature came to normal and stayed there. (Fig. 13)

This case illustrates the rapid disappearance of a hemolytic streptococcus from the blood following the administration of sulfanilamide, but

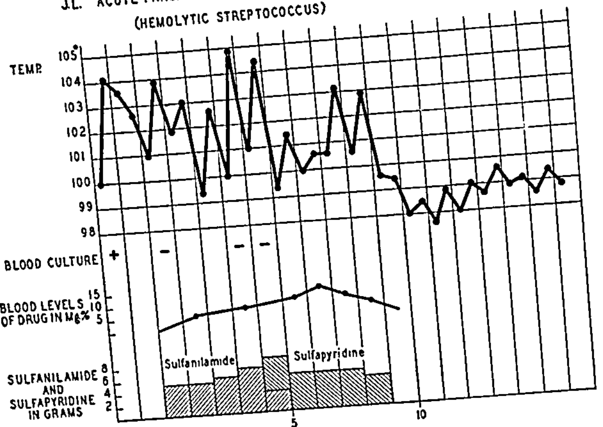


Fig. 13. Case IX. Sulfanilamide promptly cleared the blood stream and controlled the infection but fever and progressive anemia required discontinuance of the drugs. (Courtesy of Dr. George V. Browne)

a continuance of fever and progressive anemia caused by the drug. Sulfapyridine had the same effect as sulfanilamide.

Case X: R.B. Age 33. No. 694418

DIAGNOSIS Lateral sinus thrombosis. Hemolytic streptococcus septicemia.

HISTORY. The patient had been in the hospital one month before, at which time a radical mastoidectomy had been done with an uneventful postoperative course. Two days before admission, he developed a slight cold and, that evening, chills and fever. He was given sulfanilamide by his local doctor without immediate improvement.

PHYSICAL EXAMINATION. On admission, the patient's temperature was 104.4° F. and it promptly rose to 105° F. There was marked tenderness under the jugular fossa and down the right side of the neck to below the right clavicle. The ear canal and drum looked normal.

COURSE. The patient was given sulfadiazine, 9 gm. on the first day. This was increased to 12 gm on the second day. The blood culture revealed a hemolytic streptococcus. On the evening of the first day and again on the third day, he had a shaking chill. His temperature ranged between 102° and 105.8°. Blood culture was again positive. On the fourth day, the previous postauricular incision was excised and the mastoid cavity exposed. Healthy granulation tissue was curetted away and part of the lateral sinus exposed. No thrombus could be felt, but it seemed best to ligate the jugular vein in the neck. This was done. Sulfadiazine was continued. The temperature fell on the second day following the operation to 100.2°, but that evening following a transfusion he had a chill. On the third day, the chill was repeated without a transfusion and on the fourth day, he had a third chill after a transfusion, although he seemed much better. After 2 more days, his temperature fell to normal and remained there. Blood cultures were negative after the operation. (Fig. 14.)

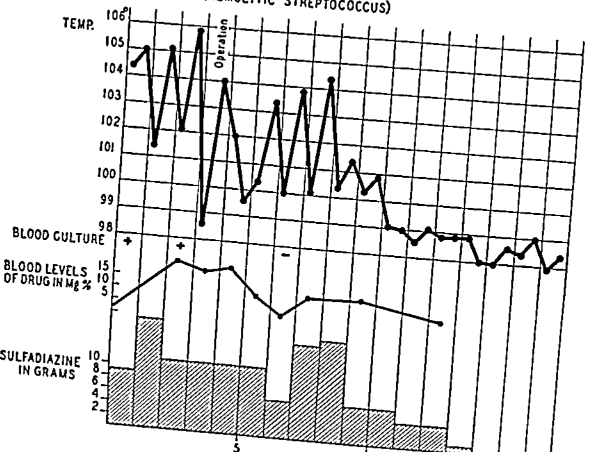
This case illustrates the recurrence of infection following an operation on a chronic mastoid with the development of a septicemia not controlled by the simple administration of sulfanilamide, but by combining chemotherapy with surgery. The recurrence of fever after the initial fall may have been due to the drug, and in this case its benefit could not be clearly established.

Case XI: C.V. Age 18 months. No. 720022

DIAGNOSIS Hemolytic streptococcus osteomyelitis of the ulna with *septicemia*.

HISTORY. The child had had frequent attacks of bronchitis and pneumonia since she was 6 months old, the last attack being 2 months before admission. Shortly after admission, she awoke from a convulsion and would not move her right arm. Next day she developed a fever and a swollen wrist. X ray showed no bone pathology. In the evening, vomiting, diarrhea, high fever, and convulsions supervened.

PHYSICAL EXAMINATION. The child threw a major convulsion on arrival at the clinic On recovery, it was noted that she failed to move the right arm.


R.B. THROMBOSIS OF LATERAL SINUS WITH SEPTICEMIA
(HEMOLYTIC STREPTOCOCCUS)
TEMP.
106°
105
104
103
102
101
100
99
98
Operation
BLOOD CULTURE
+
+
–
BLOOD LEVELS OF DRUG IN Mg %
15
10
5
SULFADIAZINE IN GRAMS
10
8
6
4
2
5

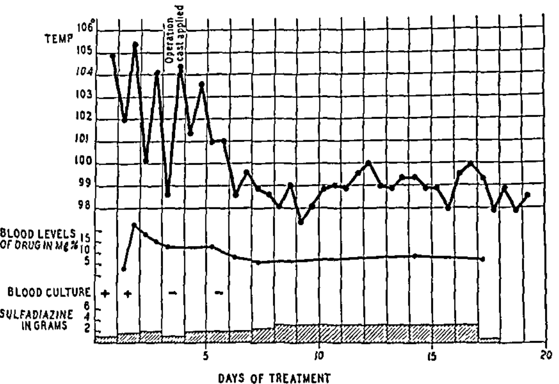


FIG. 15. CASE XI. The drug must be given credit for the cure in this case. *Operation* was of no benefit here. (Courtesy of Dr. George Humphreys)

The right wrist was hot, tender, and swollen. The temperature was 104.8°. Slight stiffness of the neck and Kernig reflex were present.

COURSE The white blood count was 31,000. Polys 77 per cent. Hemoglobin 50 per cent, red blood count 5,500,000. The spinal fluid was clear. She was put on sulfadiazine by mouth at the rate of 0.1 gm. per kilogram of body weight for 24 hours and later sulfadiazine by vein. The blood level rose to 25 mg. per cent in 12 hours. The blood culture on admission yielded a hemolytic streptococcus and next day the second culture was positive, the temperature spiking between 102 and 105°. On the third day there was a downward trend. The sulfadiazine was then given 0.5 gm. every 6 hours. This maintained the blood level at about 5.5 mg. per cent. On the fourth day, the wrist swelling was explored, but only cellulitis was found. The radius was drilled, but no pus was obtained. A cast was applied. Following operation, the temperature gradually fell and reached normal on the third day and remained there. A blood culture taken on the day of operation was negative. Later X rays showed the bone lesion to be in the ulna. The cast was removed after 3½ weeks and the wound was found to be healed. The cast was reapplied at 4-week intervals for 3 months and then discarded. The function of the arm and wrist was completely restored when last seen 8 months after onset. (Fig. 15.)

This case illustrates a hemolytic streptococcus septicemia associated with an acute hematogenous osteomyelitis, both of which were controlled by sulfadiazine. The operative procedure made little or no contribution to the recovery.

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CHAPTER XX

The Treatment of Surgical Infections with Zinc Peroxide

It is stated that zinc peroxide was first made in 1810 by Thenard,[1] who thought that he had a simple dioxide of zinc, ZnO_2. Later, in 1884, Haass[2] held that its probable formula was Zn_3O_4. In 1891 Kuriloff[3] figured it to be $Zn_2O_3H_2O$. Forcrand[4] in 1902 decided that it was probably a mixture of four compounds. The preparation now available contains about 40 to 50 per cent ZnO_2, and it is, as Forcrand thought, a mixture.

The use of zinc peroxide as an antiseptic was suggested by Elias[5] in 1903, and thereafter favorable results were reported in the treatment of several dermatologic diseases by Mayer[6] and of acute and chronic eczema and leg ulcers by Hartung.[7] Paucot[8] was impressed by its favorable effect in a number of surgical infections, especially in anal fistula. He noticed an increase in suppuration for the first few days and a subsequent decrease of suppuration followed by a rapid development of vascular granulations with a tendency to bleed. He was the first to report an unfavorable tendency of zinc peroxide to cake in the wound or in tortuous sinus tracts. He stated that he thought that certain wounds epithelialized too quickly when zinc peroxide was used, but on the whole he considered that its advantages outweighed its disadvantages.

Hochstetter[9] reported an unusual case of gangrenous ulcer of the face that responded favorably to the use of zinc peroxide and also a case of chronic endometritis. Chaput[10] thought that zinc peroxide met every requirement of an antiseptic because it was nontoxic, noncaustic, nonirritating, and could be sterilized without affecting its antiseptic value. He did not state whether or not he had demonstrated its antiseptic action *in vivo* or *in vitro* against the infecting organisms cultured from the cases that he treated, but he was satisfied by the clinical results and attributed its action to the slow liberation of oxygen over a period of hours. He used it for fresh wounds, for 'torpid' wounds, for burns, for vaginal infections, and for eczemas. He was particularly impressed by its favorable action in the postoperative treatment following the surgical removal of tuberculous bones and joints, in tuberculous ulcers, and in leg ulcers.

Vanverts[11] confirmed the observations made by Chaput. He was particularly pleased by the decrease in suppuration, the disappearance of

fetid odor, the rapid cleaning of the wound, and the prompt healing that took place following the use of zinc peroxide. He called attention to the fact that the zinc peroxide should be 'pure,' because on analysis certain ineffective samples had been found to be simply zinc oxide.

These experiences with zinc peroxide were reviewed by Laurent,[12] who determined to study the question and make it the subject of a doctor's thesis for the University of Paris. He presented some of the chemical characteristics of zinc peroxide. He considered it the ideal vehicle for the slow constant delivery of oxygen to various kinds of wounds, and believed not only that it was bacteriostatic but also that it detoxified the poisonous products of bacteria and favored the migration of leukocytes and their phagocytic action on the organisms. He demonstrated the slow but definite bactericidal or bacteriostatic action of zinc peroxide on staphylococci, streptococci, and *Escherichia coli* but did not take the opportunity to study its action on microaerophilic organisms or strict anaerobes. He added to the number of cases presented by the former authors nine of his own, including varicose ulcers, diabetic ulcers, recent accidental wounds and burns, vaginal infections, and postpartum lacerations.

All of these authors employed zinc peroxide in the form of powder, of ointment, of lotion, or of pencils. All were impressed by the deodorant action and the stimulation of wound healing. Apparently none of them studied the bacterial flora of the wounds treated, or attempted to work out a reasonable plan for the selection of proper cases for the treatment.

Perhaps it was for these reasons that zinc peroxide was not employed more generally by others. Paucot[8] hinted at certain unfavorable features of the treatment. It is probable that later experiences with it were not satisfactory because of certain difficulties in the preparation of zinc peroxide, which we shall mention later. In any event it seems to have fallen into disrepute, because nothing is found in the medical literature about it between the years 1910 and 1927, when Addario la Ferla[13] and also Pignatari[14] independently reported the successful treatment of trachoma with zinc peroxide.

I. EXPERIMENTATION

We approached the subject from the bacteriological point of view. After studying a series of chronic undermining burrowing ulcers that had been treated unsuccessfully by every conceivable method, we concluded that the etiologic organism was a microaerophilic hemolytic streptococcus, which could be consistently obtained only by anaerobic methods of bacterial cultivation.

It occurred to us, quite independently, that we were dealing with an

organism that would be inhibited by a highly oxygenated environment and we went to Dr. Hans Clarke, professor of biochemistry of the College of Physicians and Surgeons, New York, with the specific request that he suggest a nontoxic peroxide that would give off its oxygen slowly over a period of hours or days. After considering a number of possibilities, he suggested zinc peroxide, a quantity of which had been recently sent to him for experimental purposes. Its chemical action was thought to be the simple liberation of oxygen with a residuum of zinc oxide. Following his suggestion and without any knowledge of its previous clinical application, we decided to use this zinc peroxide in the treatment of the chronic undermining burrowing ulcer then available. The success of the treatment was at once apparent, and we also found that the causative organism was promptly killed after brief contact with the medicine *in vitro*. Subsequently, two other similar cases were treated with zinc peroxide with striking success. During the treatment of the latter cases it was noted that the organism underwent a striking change in the wound. It lost its anaerobic, and then its hemolytic, properties. The progeny of the original organism became a nonhemolytic aerobic green streptococcus resistant to the zinc peroxide *in vitro*. This organism persisted in the wound, but the lesion proceeded to heal in the face of its presence, behaving very much like an ordinary nonhemolytic streptococcus infection.

We reported the success of these cases and fully expected that success could be as easily obtained by others in similar cases if they used the methods that we proposed.[15]

We proceeded further to use zinc peroxide in other microaerophilic and in anaerobic infections and found that it was effective both *in vitro* against all of these organisms and *in vivo* in all cases with infections of these two types, provided that the medication could be applied to the infected surfaces. In many cases, however, the application of the medication had to be preceded by an adequate surgical procedure.

During the second year of its employment, we began to realize that the zinc peroxide was not always the same. The brilliant results in our early cases were not invariably duplicated in later cases and we began to hear that other surgeons were not having uniform success with the treatment.

We then found that the process of manufacture had been changed without our knowledge and although the manufacturer was supplying what was thought to be a better grade of material, because it was finer and whiter and suspended more evenly in distilled water, it did not give the clinical results that we wanted. It was a matter of six months after we first suspected that the 'new process' material was not up to standard that we finally persuaded the manufacturers that they must go back to the 'old process.' During this period, a considerable amount of ineffective

material had been sent out to various surgeons over the country, and a questionnaire returned by them indicated that several of their number were not particularly impressed by the efficacy of the medication.

But a return to the old process of making the zinc peroxide did not settle the matter. The manufacturers sent us various batches of material that they said were made in exactly the same way and yielded identical results on chemical analysis. We found, however, that the different batches varied greatly in their physical properties and in their clinical effectiveness. After groping around for some method of determining the difference between the effective and ineffective materials, we found that when they were suspended in distilled water in a large test tube, very obvious differences in their behavior became apparent. The effective material sedimented quickly in about 30 minutes, leaving a clear supernatant fluid. The ineffective material sedimented slowly, leaving a milky supernatant fluid. In the course of an hour, small bubbles began to appear in the sediment in the clinically effective material, whereas none at all was observed in the ineffective. After 24 hours, the sediment in the effective material was raised up by bubbles of gas (which was later shown to be oxygen), whereas in the ineffective material the sediment remained packed at the bottom of the tube, with no gas formation. At this time also the sediment in the effective material was soft and curdy, that in the ineffective was either closely packed or, in certain specimens, solidified as a plasterlike mold in the bottom of the tube (Fig. 1). Thus a test, which could be readily performed by anyone, became available to determine whether or not a given batch was effective.

It was of course necessary to go further and to determine the reasons for these apparent differences. This was done by attempting to duplicate the effective material after setting up its physical properties as a standard. We therefore measured the amount of oxygen gas produced by 5 gm. of the dry powder in 150 cc. of distilled water and noted its evolution from hour to hour over a period of 24 hours. We determined the amount of soluble oxygen in the supernatant fluid produced by 5 gm. of the powder in 100 cc. of distilled water as determined by the amount of $KMnO_4$ 0.02 normal solution that it was able to decolorize. We also determined the bactericidal action of the different batches against relatively resistant anaerobic and microaerophilic organisms. We found that the effective material produced about 10 to 12 cc. of oxygen in 24 hours and that during every hour at least 0.33 cc. was given off. We found that each batch of zinc peroxide varied from the others in the amount of soluble oxygen, but that each batch within a few hours reached an equilibrium that it maintained thereafter for several days. The ineffective batches made by the so-called 'new process' not only failed to produce free oxygen gas, but failed also to yield soluble oxygen in any great amount.



Fig. 1. A test for effective zinc peroxide. When ineffective material is suspended in water, it quickly sediments and often cakes leaving a cloudy supernatant fluid. No gas is formed in the sediment. When effective material is suspended in water, it flocculates at the bottom as a soft curd leaving the supernatant fluid clear. Gas bubbles slowly form in the curdy flocculus and lift it to the surface.

They never hardened or caked, however. The ineffective batches made by the 'old process' were either deficient in oxygen gas evolution or in soluble oxygen and frequently they showed some adhesive property in varying degree, sometimes slight and sometimes plasterlike.

We tried to find some fundamental differences between the effective and ineffective materials. There was approximately the same percentage of zinc peroxide in each, but in one the oxygen was made available (possibly by some activating catalytic agent) and in the other it remained inert. We tried to find the nature and mode of action of this activating agent by determining such factors as the hydrogen-ion concentration, the electrical charge of the particles, minute impurities as revealed by the spectroscope, sedimentation rate, gas evolution, soluble oxygen production, and the effect of various methods of sterilization.

After nearly 3 years of effort, the du Pont Chemical Company finally worked out a method for making a zinc peroxide preparation that would consistently conform to the minimum standard that we set up. Up to that time in this country, zinc peroxide had been manufactured by three firms: du Pont, Merck, and Mallinckrodt. Other chemical firms had marketed one of these three products or one of several different imported preparations. The Merck and Mallinckrodt zinc peroxides were found

ve both *in vivo* and *in vitro*, and this fact was called to the n of these firms. Merck's subsequent attempts to make a suitable : were unsuccessful and they then arranged to market the stand- product made by the du Pont Chemical Co. of Niagara Falls, N. Y., under the distinctive name 'Z.P.O.' Mallinckrodt was later also selected as a distributing agent. This can be relied upon to give consistent results both clinically and experimentally. It is now called 'Medicinal Grade Zinc Peroxide.' For the best results, it must be heated in an oven for four hours at 140° C. in quantities not larger than 250 gm. For clinical use, 15- to 20-gm. lots are most satisfactory. The heating not only sterilizes the zinc peroxide but in some manner also mobilizes the oxygen. On the basis of these experiences, the following minimum standard was determined. After sterilization, the material should meet the following tests: approximately 5 gm. of the powder are added to 50 cc. of distilled water and allowed to sediment at room temperature. The sediment should form quickly as a flocculent curd; the supernatant should be clear. Within an hour, bubbles of gas should form in the sediment and lift it up These three conditions should always prevail. The next two tests are optional. The amount of gas liberated from 5 gm. suspended in 125 cc. of distilled water at 37.5° C. may be measured by the displacement of water and should be at least 4 cc. after 1 hour and 12 cc. after 24 hours. The amount of hydrogen peroxide in the filtered supernatant fluid may also be determined and should be approximately 0 0009 per cent after 2 hours' incubation of a 5 per cent zinc peroxide suspension in distilled water at 37.5° C.[16]

With this standardized effective material we have recently made a comprehensive study of the action *in vitro* on the organisms found to be the cause of, or associated with, surgical infections.

In testing the bacteriostatic and bactericidal action of antiseptics *in vitro*, certain variables have been found to be important: the concentration of the antiseptic, the size of the bacterial inoculum; the duration of contact of the organisms with the antiseptic; and the composition of the medium Not only do different species vary in their sensitivity to any given antiseptic, but individual strains within the same species may also show variations. All of these factors have been considered in the reported study.

The basic technic in these experiments was concerned with the determination of the increase or decrease in the number of viable organisms when zinc peroxide was added to the culture medium.

In making the zinc-peroxide suspensions the medium used was a 1 per cent proteose-peptone-infusion broth made from beef heart, tubed in 10-cc. amounts. The pH of this medium was 7.4 to 7 6 except as otherwise noted The broth was boiled for 15 minutes, cooled rapidly and

immediately inoculated with the test organisms. In certain experiments, horse serum or sheep's whole blood was added to the broth.

The required amounts of zinc peroxide were weighed out under sterile precautions from batches previously sterilized for clinical use. At times, the addition of zinc peroxide to the medium tended to increase the alkalinity. No lot of zinc peroxide was used for these tests in which the pH of the zinc-peroxide suspensions was more than 8.2 at the beginning of the experiment. In some instances this necessitated using an acid broth in making the 10 and 20 per cent suspensions.

The standard inoculum was 0.5 cc. of an 18-hour culture of the test strain. In determining the effect of varying the size of the inoculum, decimal dilutions of 0.5 cc. of the 18-hour culture were also used.

The number of viable organisms per cubic centimeter in the seeded tubes before the addition of zinc peroxide was determined by decimal dilution series in 1 per cent dextrose broth, and platings on blood agar of 0.1 cc. of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions. The seeded zinc-peroxide suspensions, together with a control tube for the normal rate of multiplication of the organisms, were incubated at 37.5° C. At definite intervals, the number of viable organisms was determined, as previously described.

The detailed results of these studies may be found in the report in the *Annals of Surgery*.[17] They may be summarized as follows: The organisms highly sensitive to the bactericidal action of zinc peroxide are: hemolytic streptococci—aerobic, anaerobic and microaerophilic; pneumococci; the vegetative forms of the anaerobic sporebearing bacilli—*Clostridium welchii, C. tetani, C. histolyticum, C. sporogenes, C. novyi, C. sordellii,* and *C. septicum;* the anaerobic nonsporeforming bacteria—nonhemolytic streptococci, both anaerobic and microaerophilic; *Bacillus fusiformis* and *B. necrophorus* (see Table I).

The organisms that proved to be relatively resistant were: *Streptococcus viridans, Staphylococcus aureus, Escherichia coli, B. proteus,* and *Pseudomonas pyocyanea.*

The spores of the anaerobic sporebearing clostridia were also relatively insensitive. Of the species tested, the spores of *C. welchii* and *C. tetani* were more susceptible than those of the other bacilli in this group.

The hemotoxins of streptococcus and *C. welchii* were destroyed or inactivated by zinc peroxide *in vitro,* as shown in Table II.

The active treatment of surgical infections with zinc peroxide should await the bacteriological determination of the flora of the wound and should be used only if those organisms are found that are susceptible to peroxides—namely, the anaerobes, the microaerophiles, and certain aerobes, including the ordinary hemolytic streptococcus. When an infection with these organisms has become established, particularly in cases of

TABLE I

DIFFERENCES IN THE SUSCEPTIBILITY OF INDIVIDUAL STRAINS OF HEMOLYTIC STREPTOCOCCUS TO THE BACTERICIDAL ACTION OF ZINC PEROXIDE AFTER FOUR AND TWENTY-FOUR HOURS INCUBATION AT 37.5° C. IN A 0.5 PER CENT SUSPENSION IN INFUSION BROTH

Plate Colony Counts: 0.2 cc -10^{-2} Dilution of Experimental Tubes
48-hr. Readings

STRAIN	EST. NO. OF BACTERIA AT THE START *	CULTURE CONTROL AFTER		0.5 PER CENT ZnO_2 IN MEDIUM AFTER	
		4 Hrs.	24 Hrs.	4 Hrs	24 Hrs.
Anaerobic					
D-	4,900	++++	++++	0	0
K-	2,950	++++	++++	1	0
Microaerophilic					
L- †		++++	++++	0	0
M- †	2,100	++++	++++	0	0
Sa- †	3,000	++++	++++	130	0
Se- †	4,300	++++	++++	21	0
Ro-	5,300	++++	++++	108	0
R- ‡	4,450	++++	++++	100	0
				40	0
Ma-	2,400	++++	++++	257	0
Ke-	1,900	++++	++++	51	0
Ko-	3,000	++++	++++	320	0
St-	2,800	++++	++++	43	0
Be-	4,100	++++	++++	52	0
Si-	5,000	++++	++++	97	0
At-	1,980	++++	++++	42	0
Aerobic					
C-	2,700	++++	++++	250	0
En-	2,400	++++	++++	360	0
Ei-	2,100	++++	++++	250	0
	4,900	++++	++++	300	0
Do-	3,800	++++	++++	600	0
F-	2,000	++++	++++	250	0
Sac-	5,200	++++	++++	680	0

* Calculated from platings of decimal dilutions of control tube before incubation.

† These strains were growing only anaerobically on solid media when the tests were made.

‡ Represents one test with different lots of zinc peroxide

TABLE II

EFFECT OF ZINC PEROXIDE IN VITRO ON STREPTOCOCCUS AND CLOSTRIDIUM WELCHII HEMOTOXIN

HEMOTOXIN	ORIGINAL TITER	TEMP. EXPT.	TIME OF ACTION	CONTROL TITER	TITER TOXIN + ZnO_2			
					20%	10%	5%	1%
S. hemolyticus	1:128	23° C.	1 hr	1:128	0	0	1:8	1:32
			2 hrs.	1:128	0	0	1	1:16
			3 hrs.	1:64	0	0	1	1:8
S hemolyticus	1:128	23° C.	4 hrs.	1:128	0	0	1	1:16
			12 hrs	1:128	0	0	0	0
C. welchii	1:256	22 6° C	1 hr.	1:256	0	0	1:32	1:64
			2 hrs	1:256	0	0	1:16	1:64
			4 hrs	1:128	0	0	1:4	1:32
			18 hrs	1:128	0	0	0	0
C welchii	1:256	37 5° C	2 hrs.	1:128	0	0	1:2	1:4
			4 hrs.	1:128	0	0	1	1
			12 hrs.	1:128	0	0	0	0

tetanus or gas gangrene, there must be a preliminary surgical procedure *that will render it possible* to apply the medication to the infected tissues in order that the main focus may be removed and the medication have a chance to act upon the residual infection. It is possible in many cases to *make a clinical diagnosis of anaerobic infections, but anaerobic methods* of bacterial cultivation are essential for an accurate determination of the nature of the infection.

II. CLINICAL INDICATIONS FOR THE USE OF ZINC PEROXIDE

Zinc peroxide is particularly indicated in those foul-smelling infections of dental origin that occur in the face or neck, in which the anaerobic nonhemolytic streptococcus and the spirochetes and fusiform bacilli of Vincent and Plaut are the etiological agents.[18-20] Likewise, in neck infections from perforations of the esophagus that are particularly virulent and necrotizing in their effect. Lung abscesses and chronic empyemas *following lung abscess are particularly amenable to treatment with zinc* peroxide, and the gratifying deodorant effect is almost immediately noted and appreciated by the patient and the whole ward. Chronic abdominal sinuses, either fecal fistulas or those arising superficially and invading the

deeper tissues, are frequently due to susceptible organisms of intestinal origin. Progressive gangrenous infections of the skin due to a synergistic bacterial action or chronic undermining burrowing ulcers of the non-gangrenous type (see frontispiece) are indications for the use of zinc peroxide after the necessary preliminary surgery that will permit close application of the medication.[21, 29] Human bites fall into the same category.

Gas gangrene or tetanus resulting from gunshot wounds or street injuries should be treated with zinc peroxide after the involved areas have been surgically debrided.

Zinc peroxide has been used successfully as a prophylactic in accidental and surgical operative wounds where contamination with anaerobic and microaerophilic organisms was suspected or certain. As one would expect, the prophylactic use of this material is more logical than the active treatment, because the organisms have not yet invaded the tissues, but are merely on the surface. The application of zinc peroxide to the wound surfaces gives intimate contact with the organisms so that they are either killed promptly by the action of the zinc peroxide or are prevented from gaining a foothold and multiplying in the tissues. Then they are either destroyed by leukocytes or are washed out of the wound. In such cases, as in the treatment of active infections, the zinc peroxide must be applied to every part of the wound surface, because its action does not extend very far from its actual presence. Clinically, it acts very much the same as it does on a blood-agar plate. If a drop of zinc-peroxide suspension is placed upon the surface of blood agar that has been evenly seeded with susceptible bacteria, after incubation a narrow zone about $\frac{1}{16}$ of an inch wide will be seen around the drop; in this zone there will be no growth of bacteria, whereas the rest of the plate will be evenly covered with luxuriant growth.

Inasmuch as anaerobic and microaerophilic organisms are prevalent throughout the alimentary canal and in the vagina, zinc peroxide is indicated prophylactically before all operations on the mouth, such as plastic procedures or the removal of tumors of the mucous membrane. One is surprised by the lack of inflammatory reaction of the mucous membrane when it is used as a mouth wash before and after such procedures. No untoward results have followed the ingestion of zinc peroxide when it is so employed. It should be used before dental extractions as a means of preventing those foul infections so frequently seen in the tooth socket and in the neck after such dental work. In these infections nonhemolytic anaerobic streptococci and spirochetes and fusiform bacilli are active, probably in symbiosis. For the same reason, zinc peroxide is indicated in all human bites. In the latter cases, the wounds should be opened widely and surgically debrided so that contact may be had with the fullest depths of the wound. The destructive action of these infec-

tions if they gain a foothold—with their tendency to invade bones and joints and tendons—is well known. If zinc peroxide were used before and after tonsillectomies, the extensive sloughs and the occasional fatal septicemia or lung abscess that are the bane of the throat specialists would, we believe, be greatly diminished. Operative or accidental wounds of the esophagus may safely be left open and packed with gauze soaked in zinc peroxide suspension, the dressing being renewed daily. Colostomies or cecostomies should be surrounded by packing soaked in zinc peroxide, both preliminary to and after their opening. Recently, one of my confreres had a patient who developed an extensive gas gangrene of the abdominal wall when this was not done. Fortunately excision followed by the application of zinc peroxide controlled the infection. After excision of an anal fistula or fissure, the tract should be packed with gauze soaked in zinc peroxide suspension.[30] The lack of inflammatory reaction and pain and tenderness will be noted both by the doctor and patient when this is used After hemorrhoidectomy the anal ring should be similarly packed during the postoperative period of enforced constipation. The patients so treated have almost invariably stated that pain was surprisingly absent. In recent years, gauze impregnated with zinc peroxide in a carbowax base has entirely replaced iodoform gauze in our practice. Gauze so treated has been very useful in the primary treatment of burns.[31]

For several years the author has been using zinc peroxide as a preparation for the vagina preliminary to hysterectomy or the plastic procedures on the female perineum, and we have had a feeling of security against infection caused by the anaerobic organisms and the hemolytic streptococcus that are the chief offenders in infections arising in these regions. Surgeons working in the field of cancer have found it exceedingly valuable in controlling secondary infections.[32-4] Gunshot wounds are so contaminated by anaerobic organisms that zinc peroxide is indicated as a prophylactic to prevent the development of infection. Similarly, severe civilian accidental wounds and compound fractures are also amenable to this treatment.[35 f]

III. THE APPLICATION OF ZINC PEROXIDE

There are three essential features in the use of zinc peroxide: (1) the material must be an active oxygen liberator, according to the test described above (page 591); (2) contact must be obtained with every part of the infected surface; and (3) the dressing must be kept moist.[38, 39]

When the time comes to use the material, the powder is mixed with approximately an equal quantity of sterile distilled water. This is best accomplished by the use of an 'asepto' syringe. The material should be mixed until it has a creamy consistency and then should be applied with

the syringe to the surface of the wound, care being taken that every part of the wound's surface is covered with a thin layer of the material. Special care should be taken to apply it under any undermined skin margins or into any deep sinuses. The wound should then be covered with a thin layer of absorbent cotton soaked in the material or with two layers of fine-meshed gauze likewise soaked in the suspension (Figs. 2-5).

Over this, to keep the dressing wet, should then be placed a half-inch layer of absorbent cotton soaked with sterile distilled water and this in turn covered with several layers of fine-meshed gauze impregnated with vaseline or better still with zinc-oxide ointment. This should be so applied that it seals the dressing and effectively prevents evaporation (Figs. 6-7).

After 24 hours, the dressing should be removed *en masse*. It will not adhere to the wound if it is still moist. The exudate and old zinc peroxide should then be washed away from the surface of the wound by means of a stream of saline from an irrigating can. Slough that is easily removed may be gently lifted out of the wound or cut off, but there should be no rubbing of the wound or other trauma to the surface. The dressing should then be reapplied as before.

The wound will look gray and inert for 3 or 4 days. Particles of zinc peroxide cling to the tissues. One is struck by the lack of any inflammatory reaction or evidence of irritation. After 4 days, granulations begin to appear and within a few more days the wound will become red with active healing, and new epithelium will begin to grow in at the margins. If it improves in some places but not in others, one can be pretty sure that there is not adequate contact in those other places. Then greater care must be observed in getting contact in these areas, and if that is impossible the affected areas must be excised.

Cultures should be taken from the wound two or three times a week. As soon as the anaerobic organisms are no longer found on two successive cultures, secondary closure or skin grafting may be considered. The zinc-peroxide treatment should always be checked by studies in a laboratory thoroughly equipped to do both anaerobic and aerobic bacteriology. One case so treated is illustrated in Figures 8-11.

IV. THE LIMITATIONS OF ZINC PEROXIDE THERAPY

Although this agent is bactericidal as well as bacteriostatic and is apparently without injurious action on the tissues, it can only act i comes in contact with the infecting agent, and being insoluble it only local surface action. It can be used therefore only where its resic zinc oxide can be removed after the zinc peroxide has served its purp Furthermore, it must be kept moist, for if it dries, its action stops



Fig. 2. Irrigation of the wound with saline. All loose particles are removed from the wound in this manner.



Fig. 3 The sterile tray with dish, irrigating can, and basin. Pouring zinc peroxide from the tube in which it has been sterilized Distilled water is in the asepto syringe.



Fig. 4. Mixing the zinc peroxide with distilled water until it attains a creamy consistency.

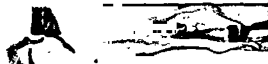




Fig. 5. The wound surfaces are flooded with the zinc peroxide suspension, by means of an asepto syringe.

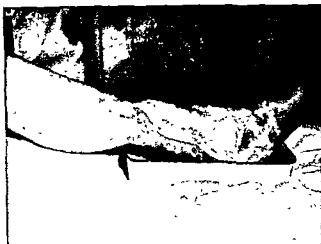


Fig. 6. A thin layer of cotton soaked in zinc peroxide has been laid over the surface of the wound and this in turn covered by a thick layer of cotton wet with distilled water.



Fig. 7. The wound is then sealed with several layers of fine meshed gauze impregnated with vaseline or better still with zinc oxide ointment and so applied that it effectively prevents evaporation.



Fig. 8. An extensive undermining, burrowing infection of both buttocks of 14-years duration before treatment.

Fig. 9 The lesion has been excised and the infection has been brought under control with zinc peroxide. The area is now ready for skin grafting.



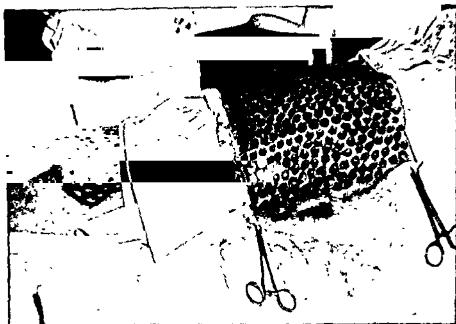


Fig. 10. Deep Davis skin grafts have been cut from the back and placed on the granulating wound of the buttocks.

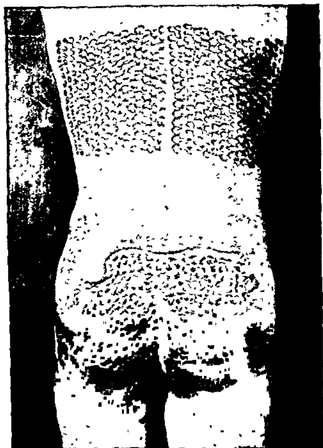


Fig. 11 The final result is satisfactory with the infection controlled and all scars soft and pliable.

it may mechanically injure the granulating surface by rubbing against the wound.

Attempts have therefore been made to suspend it in a vehicle in which it would not dry or cake. Unfortunately, in any oil or greasy medium it is inactive, probably because the zinc-peroxide particles become coated with a layer that prevents the wound secretions to activate the drug. The best liquid medium for the desired purpose is a 2 per cent polyvinyl alcohol, which is a mucilagenous liquid holding the zinc peroxide in suspension better than water. This however will dry when it is used on the surface and must be kept wet. It is therefore not much better than the watery suspension. When zinc peroxide is used in the colon for the treatment of ulcerative colitis, the polyvinyl alcohol is of real advantage and prevents the medication from forming a hard mass in the lower rectum when the dehydrating function of the colon absorbs the water from the stool. It may be used as a retention enema for the milder forms of ulcerative colitis or through a cecostomy for the more severe forms.

Recently, both Reid and Altemeier[40] and the author have independently developed a carbowax base containing no water but readily water-soluble. This base—made of hexa-ethylene glycol, 35 parts; and carbowax 1540, 45 parts—will maintain the potency of zinc peroxide 10 to 20 per cent for several months. For easy application the ointment may be incorporated into fine-meshed gauze similar to vaseline gauze. This is particularly suitable for the prophylactic treatment of burns, bed sores, or other extensive surface infections caused by susceptible organisms. The ointment melts at body temperature and when it is applied to a surface infection, it permeates into the interstices of the wound. It may therefore be used in almost any situation in which the water suspension was formerly used, but it is not quite as active in the production of oxygen as the former preparation. Altemeier[41] has found that the oxygen evolution is considerably enhanced by the addition of calcium peroxide one part to ten of zinc peroxide.

Many of the infections formerly responding only to zinc peroxide yield to the sulfonamides or to penicillin, but the chronic undermining burrowing ulcer, the lesion that first brought zinc peroxide to our attention as a therapeutic agent, is still in most cases resistant to these newer drugs and still requires treatment with zinc peroxide to bring it under control.

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The Bacteriophage Therapy of Surgical Infections

TWORT[1] is generally credited with making the first 'bacteriophage.' While working with smallpox vaccine virus, Twort discovered on his culture plates some contaminating colonies of micrococci. Among these colonies, he observed glassy areas where the micrococci were undergoing dissolution. He was able to carry over from these glassy areas a substance that continued to have this disintegrating effect on subsequent cultures of the micrococci. He found that this active substance would pass through a filter, and he offered the theory that it was in the nature of a filtrable virus He later made some similar observations with intestinal organisms of the typhoid-colon group, but he was unable to follow up these interesting leads.

Two years later, d'Herelle became the chief observer of the phenomenon and the stalwart advocate of its clinical application for the control of infection. He claimed priority of discovery, referring to observations made by him in 1909. He questioned whether Twort and he were describing the same phenomenon. Certainly if the two reports are compared, there is very real doubt that Twort was dealing with bacteriophage.[2] While studying an epidemic of dysentery, d'Herelle observed that if a filtrate were made from the stool of a patient who had recovered from dysentery, a drop of filtrate added to a turbid pure culture of the Shiga dysentery organism would clear the culture within a few hours of incubation[3] He also noted that a drop of this cleared culture added to a fresh turbid culture would clear that also, and that this could be repeated over and over again, each clearing representing a very rapid amplification, multiplication, or propagation of the agent causing the phenomenon. D'Herelle believed that the only possible explanation of this behavior was the activity of a living organism. In 1931, he said, 'The bacteriophage corpuscle is a living ultra-microscopic being as is proved by the fact that this corpuscle dissolves bacteria through the agency of a ferment which it secretes. The secretion of a ferment implies a metabolism and this is an essential character of living beings. A bacteriophage is therefore of necessity a virus, a parasite of bacteria.'[4]

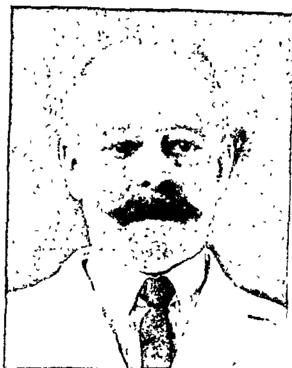


FIG 1. D'HERELLE. Dr. Felix d'Herelle was the co-discoverer of the phenomenon of bacteriophagy and the chief exponent of bacteriophage therapy. (*Courtesy of* Dr George H Smith, Professor of Bacteriology, Yale University, New Haven, Connecticut)

D'Herelle made his discovery during an epidemic of dysentery and promptly brought the resources of his laboratory to bear upon the clinical problem at hand. He tried to fit this phenomenon into the whole system of resistance and immunity to infectious diseases and attempted to make general application of the principles involved to all kinds of medical and surgical infections. Thus he reported miraculous results in the control of dysentery in the Sudan, of cholera in Punjab, of plague in Egypt, and of staphylococcal infections in Paris. D'Herelle claimed that a study of convalescent patients would show that recovery is frequently if not always associated with the development of a bacteriophage. This was easy to demonstrate in many cases of dysentery but not so easy with staphylococcal infections. D'Herelle believed that epidemics come to an end by the gradual development in the affected community of a relative increase of bacteriophage over the bacteria causing the epidemic, and he conceived the idea of the 'infectiousness of health,' meaning the wide dissemination to all kinds of potent phages. He came to the conclusion that 'bacteriophage *exists in the intestinal contents of all healthy individuals*, where it grows at the expense of the saprophytic bacteria, of *E. coli* in particular, which are daily ingested with the food. Thanks to

its faculty of adaptation, the normal intestinal bacteriophage becomes able to parasitize foreign bacteria which may become implanted not only within the intestine but in any organ whatsoever, for experiment shows that the bacteriophage passes readily into the circulation.'[4] He believed that if any focus of infection developed anywhere in the body, phage in dilute amounts coming from the intestine and circulating through the blood would sooner or later attach itself to the bacteria at the site of the infection and begin at once to propagate itself until the infection came under control. Although this might happen naturally, d'Herelle advocated the administration of the appropriate phage by the best route to get it in contact with the bacteria in order to hasten this natural process—by mouth for intestinal infections, locally for skin and subcutaneous lesions or bladder infections, and systemically for internal infections that could only be reached by the blood stream. Thus he became convinced that 'treatment by bacteriophage has been the specific treatment par excellence since it leads to recovery through a mechanism identical with that of natural recovery.'[4]

D'Herelle recognized the fact that the phenomenon of lysis by bacteriophage was different in the test tube from its behavior in the body, for *in vitro* there are only two factors at work—the bacteriophage and the bacteria, while in the body the host plays an important part either aiding in the destruction of the bacteria by means of phagocytosis, bacteriolysis, and so on, or interfering with the action of the phage by adsorption, rapid elimination, or antiphage inactivation. The outcome in any given case would depend upon the relative strength of all of these forces. For the best results d'Herelle advocated (1) a potent bacteriophage, (2) a recently isolated and propagated phage, and (3) intimate contact between the phage and the organism.[4]

These provisions are not easy to fulfil and the clinical application of this new principle met with frequent failure, but d'Herelle remained firm in his conviction that it would eventually win universal approval.

The commercial exploitation of bacteriophage flooded the market with impotent preparations, which deteriorated with the passage of time, and which were used by practitioners who had no facilities for determining what organisms were causing the infections they were treating or whether the infections were susceptible to the lytic action of the phage they were using.

The only workers in this field that had any opportunity of fulfilling the prerequisites necessary for success were those who had available both the clinical material and the laboratories, which permitted close collaboration on the problems at hand.

D'Herelle's reports aroused great interest among bacteriologists and

immunologists, particularly those interested in filtrable viruses, and during the 1920's and 1930's, an increasing number of reports appeared in the medical literature, reaching an all-time high of 75 or more articles in 1939. Space does not permit a complete review of this voluminous literature here, but the subject has been simplified by the fact that three summaries have been published, one by Eaton and Bayne-Jones,[5] one by the author[6] independently in 1934, and one by Krueger and Scribner in 1941.[7]

I. THE NATURE OF BACTERIOPHAGE

The reports that have appeared fall into three main groups: (1) those written by laboratory workers who have investigated certain phases of the chemical, physical or biological behavior of the lytic principle and have been interested in the Twort-d'Herelle phenomenon as such, rather than in its clinical application; (2) those written by clinicians who have frequently used commercial phages in the treatment of infections without adequate controls or accurate knowledge of the bacteria causing the infections, and (3) those written by individuals or groups who had close contact both with the clinic and the laboratory and were able to check the cultures of the cases under treatment, determine susceptibility of the organism, and properly evaluate their results. The largest portion of the literature falls into the first group. Many workers gave their time and attention to the question of the viability of the active principle. Some of the earlier workers accepted the assertions of d'Herelle that phage was a living organism in the nature of the filtrable viruses. It behaves in many respects like several of the filtrable viruses, the viability of which has not been questioned. To the support of d'Herelle's hypothesis came Burnet,[8,9] Asheshov,[10-12] Jadin[13] and others, whereas Bordet,[14] Krueger,[15-21] Northrop,[22] Hadley,[23] Kendall[24,25] and others contended that evidence clearly indicated that it was not a living organism. Bronfenbrenner[26] avowedly set out to prove that it was not living, but finally had to admit that he could not demonstrate that beyond question.

Although Bronfenbrenner believes that bacteriophage may be derived from the bacterial protoplasm, he has never been able to induce the spontaneous appearance of phage in cultures of various types known to be free from phage. As he has shown that phage may be carried by a culture for a long time without revealing itself, he emphasizes that reports of the spontaneous origin of phage in old cultures must be interpreted with this fact in mind.

Bronfenbrenner admits that the bacteriophage principle is antigenic and that when injected into animals it produces an antibody independent of the antibodies produced by the bacterial antigens that may be present

in the filtrate containing the phage. When the latter antibodies are adsorbed from the serum the presence of the phage antibody in the serum may still be demonstrated.

In Bronfenbrenner's opinion, one of the chief arguments against the theory that the bacteriophage is a viable virus is the fact that he has been unable to detect by means of a microspirometer any evidence of respiration during a period of ninety-six hours by a bacteriophage that contained ten[12] active units per cubic centimeter. He contends that if the bacteriophage respires at all its respiration must be 10,000 times slower than the respiration of an equal number of bacterial spores, or it would be detected by the microspirometer in that length of time.

Other workers have taken issue with Bronfenbrenner on a number of points cited. Asheshov and his co-workers[10-12] are convinced that the bacteriophage is a living virus and believe that the plaques or 'clearings' that appear on solid media represent colonies of bacteriophage. They claim that on the basis of morphology and behavior these colonies may be classified into several groups. Pure lines of bacteriophage may be obtained by repeated fishing of the plaques just as pure strains of bacteria are obtained.

Experiments carried out by Eaton[27] yielded results quite contrary to those of Bronfenbrenner with respect to the respiration of bacteriophage. Eaton found that a culture of staphylococcus-lysing bacteriophage, containing a negligible number of living bacteria, continued to give off carbon dioxide and to take up oxygen for several hours. He therefore concluded that either the bacteriophage or some product of its action respires.

Burnet,[8, 9] in a series of excellent articles, took up a number of these points. He was able to classify phages not only by the variations in the appearance of the plaques but also by the antigenic differences of diverse races and their behavior with respect to the various dissociated phases of certain strains of bacteria. Both Burnet and Asheshov found phages that acted only either upon rough or upon smooth variants of certain cultures; and Burnet, by the use of the appropriate phage, was able to clear of one or the other form, cultures containing both rough and smooth forms. In each case, the resistant form was the nonlysable variant. Burnet was able to classify certain coli-dysentery phages by the 'resistance' technique of Bail[28] By studying the resistant forms that developed following the application of a series of phages to a single strain of *Bacillus coli communis,* he was able to divide them into four groups. On the basis of serological differences, he divided the coli and dysentery phages into twelve groups He differed with d'Herelle, who has argued for the unity of the lytic principle, but agreed with him that bacteriophages

are living viruses. He believed that the serological differences support the theory that bacteriophages represent a heterogeneous assemblage of independent viruses parasitic on or living symbiotically with bacteria. He was able to correlate the serological classification with the findings of certain biochemical tests especially in regard to the rate of photodynamic inactivation by methylene blue, the ability of the phages to lyse in the presence of citrate, and the rate of their inactivation by strong urea solutions.

Hadley, who has been particularly interested in bacterial 'dissociation,' has tried to correlate this dissociation with the changes taking place in cultures whose growth is modified but not destroyed by bacteriophage. He expressed the view that bacteriophage action is far from being a parasitism of bacterial cells by a foreign filtrable virus, but merely one aspect of the large problem of microbic dissociation and probably involves a reproductive and pseudolytic mechanism. Hadley and Jiménez[21] found that the process is reversible. Phage plus culture produced variants. Likewise, in cultures in which variants were produced by other means, bacteriophage developed spontaneously.

From a study of the size of the bacteriophage unit, Rahn[29] concluded that *the bacteriophage cannot contain the complete growth mechanism* of a bacterial cell and its protoplasm must be similar to that of its host. He estimated its size to be about that of a gene, and suggested that the bacteriophage is an unbalanced protoplasm molecule of the bacterium that has lost adaptation to the regulating mechanism in the cell.

Colvin[30, 31] demonstrated that bacteriophage is often present in the air of a laboratory in which it is being studied and that therefore cultures of bacteria may be easily contaminated by it and the 'spontaneous generation' of bacteriophage must be discounted. After a *Bacillus megatherium* bacteriophage was sprayed about a room with an atomizer, he observed the formation of a number of plaques on exposed Petri plates freshly seeded with *B. megatherium*. Plaques appeared on the plates as late as eighteen days after the spraying. In a draughty hall, particles of phage were deposited on exposed Petri plates at a distance of 30 meters.

Sertic[32] studied plaques of bacteriophage having an outer zone. He was able to demonstrate that in this zone, lysins free from phage were present. This observation seemed to indicate that certain phages are capable of producing diffusible lysins that are not transmissible in series but are antigenic and produce antibodies preventing the lytic action of the bacteriophage.

As a result of his long series of experiments, Krueger[19] became firmly convinced that phage is not a living organism. It had been thought that phage could be propagated only by contact with a living cell and that

lysis would occur when the numerical relationship of 'activity units' to the bacterial bodies was 100 to 1, but Krueger showed that by the proper changes in temperature, salt content, and hydrogen-ion concentration, the growth of bacteria could be held in abeyance while the phage continued to increase. He also found that phage could be propagated by adding it to an ultra filtrate of bacteria without bacterial bodies' being present.[19] He concluded that 'phage is fabricated in much the same way that other enzymes are, namely, by the cellular synthesis of an inactive precursor which is later changed to the active form—like trypsinogen, developed by the pancreas in the inactive form, is subsequently converted to the enzyme trypsin in the small bowel or trypsinogen in solution can be autocatalytically transformed into trypsin by the addition of a small amount of trypsin to it.'[7]

Krueger also demonstrated that phage could be inactivated by heat,[17] by $HgCl_2$,[15] or by KCN,[16] and after a period of time (long enough to kill most living things) it could be activated again by appropriate means designed to offset the inactivating substance. All of these experiments tended to show that the phage was not a living thing.

Bordet[14] believed bacteriophage to be a way that early death and autolysis of the culture is brought about. Hadley[23] believed that it is an inanimate substance that causes dissociation of the bacteria from visible forms to an invisible filterable stage and that this process is reversible as indicated by later secondary growth. This discussion and his own experiences with phage led Herb[33] to the belief that the bacteriophage serves as the connecting link between the one-celled organism and the inorganic world, thus making evolution a continuous and uninterrupted process developed by forces that can be followed up scientifically.

In spite of the strong arguments brought forward by the advocates of the nonviability theory, we have a recent demonstration by the electron microscope by Luria, Delbruck, and Anderson[34, 35] that is very convincing evidence on the other side of the argument (Figs. 2-5). They have pictured *Escherichia coli* cultures and two strains of coliphage, which they called the alpha and gamma viruses. What are interpreted as the bacteriophage particles are seen as oval bodies lined up on the surface of the bacterial cells. Their size conformed closely with the size as measured by Luria and Exner's experiments with radiation and phage.[36] To quote from their report:

Micrographs of suspensions of virus alpha and of virus gamma show the presence of particles of characteristic shape and size, specific for each strain. Regarding the identification of the particles visible on these pictures, with the viruses rather than with inert bacterial constituents, we note. 'the two viruses were grown at the expense of the same host; the same bacterium then produces

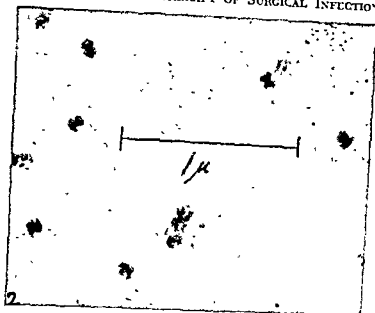


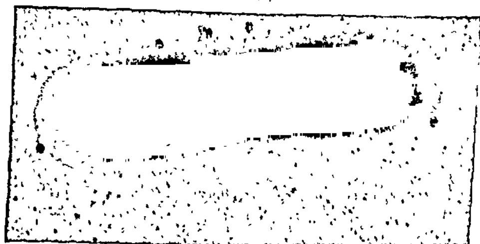
FIG. 2. Particles of bacteriophage showing the characteristic head and tail. (Courtesy of *Dr. S. E. Luria*)

particles of type alpha if acted upon by virus alpha, and particles of type gamma if acted upon by virus *gamma*.'

The particles of virus alpha have a round 'head,' 45-50 micromu in diameter and uniformly dark in the micrographs; that means uniformly scattering for 60 kV *electrons*. To this round 'head' is attached a 'tail,' about 150 micromu long and *not more than 10-15* micromu thick. The tail appears either straight or slightly curved.

The particles of virus gamma present a very peculiar aspect. To an oval *head, 65 x 80* micromu, a straight tail, 120 micromu long and 20 micromu

FIG. 3 *E. coli* + bacteriophage. 15 minutes contact. Dividing bacterium with 16 adsorbed particles of virus visible on its edge, × 16,500. (Courtesy of Dr. S E. Luria)



II. THE CLINICAL APPLICATION OF BACTERIOPHAGE TO SURGICAL INFECTIONS

But the argument regarding viability is of secondary importance to the question of the clinical usefulness of phage. It is extremely hard to evaluate many of the clinical reports of individual cases or small series in which the bacteriology was incomplete or absent. Only in those institutions in which the laboratory can stand back of the clinical studies can any significant results in bacteriophage therapy be obtained. Such a co-operative effort has been carried on for the last twelve years at the Presbyterian Hospital (New York City)—with the close collaboration of the bacteriological research laboratory of the Department of Surgery, Columbia University—by Dr. Helen Jern and the author and our associates Balbina Johnson, Olga Mordvin, Agnes Cooper, Catherine Capraro, Harold Harvey, Alfred Longacre, Edward Howes, Salvatore Luria, and John Goodner. Our studies have been the basis of eleven papers that have appeared in several different journals.[34-6, 38-46] These will be the basis for the subsequent discussion in this chapter.

In evaluating the clinical results of bacteriophage, Krueger[7] has pointed out that the filtrate, obtained from the lysis of bacterial cultures by bacteriophage, contains not only the phage but the broken bacterial bodies and their dispersed contents, as well as the elements of the medium in which the growth of bacteria and phage and the lysis has taken place. If this filtrate is injected or applied to the animal or human body and if there is any benefit obtained in the treatment of infection, it might be due to one or more of the following factors:

1. The *in vivo* lysis of the bacteria causing the infection
2. Nonspecific protein shock
3. Local immunization of a general nature
4. Specific immunization by the bacterial products
5. The promotion of phagocytosis
6. The antivirus of Besredka
7. Bacterial dissociation with less virulent variants

Krueger believes that there is little evidence that there is ever any *in vivo* bacteriolysis by the phage, and that if it does exist it is the least important of the factors. This is quite at variance with the belief of d'Herelle, who at the same time set down the prerequisites for success mentioned before, namely: (1) a phage able to lyse the culture of the organism causing the infection, (2) a freshly made phage preparation, (3) close contact between the phage and the bacteria.

Our work wholly substantiates the necessity of these criteria, but we

have come to require a stricter criterion of potency than the one advocated by d'Herelle. He considered a phage sufficiently potent if it completely cleared the culture. We believe that in order to obtain the highest possible measure of success, the phage must not only be able to clear the turbid culture of the causative organisms but it must also be able to kill it. We have found that there is a great deal of difference between lysis and death, and lysis alone cannot be a satisfactory criterion of the potency of the phage. We believe that this is the most important element in the successful clinical use of phage and that all the other factors are of minor importance. In fact such unpleasant results of treatment as protein shock should be carefully avoided if possible. Moreover, we believe that propagation of phage may take place in the blood stream following systemic administration and in the lesion following local application.[15] The recent experiments of Dubos and Straus[17] lend weight to this opinion.

III. USES OF BACTERIOPHAGE IN SURGICAL INFECTIONS

The experiences that have led us to these views regarding the use of bacteriophage in surgical infections follow.

A. Phage Therapy in Peritonitis

We first took up the question of phage therapy in the course of our study on peritonitis. We demonstrated protection of mice against intraperitoneal injection of a virulent strain of *E. coli* by the simultaneous or later injection of a potent *E coli* bacteriophage. This is indicated by one of our protocols. (See Table 1.)

The clinical application of phage in cases of human peritonitis met with equivocal success, and the explanation seemed to lie in the fact that the disease was often due to a mixture of organisms with many of the *E. coli* groups resistant to our phages.

B. Phage Therapy in Urinary-Tract Infections

In many cases of cystitis, the causative organism is one of the coli group. These are often active in pure culture and the test of susceptibility to phage may be carried out easily. Then contact is readily obtained either by local application through the urethra or by intravenous or intramuscular injection If general symptoms of chills and fever with or without bacteremia indicate kidney pelvis or ureters or the kidney parenchyma to be involved, local application will not obtain contact. Then the therapy must be given systemically. But this must be done cautiously, for very small amounts may cause a sharp reaction with chill and a spike of fever This reaction is probably due to bacterial split

TABLE I

SHOWING THE PROTECTIVE ACTION OF *E. coli* BACTERIOPHAGE IN THE TREATMENT OF EXPERIMENTAL *E. coli* PERITONITIS IN MICE.

NO	DOSE IN MILLIONS OF E. COLI	USED FOR TREATMENT		INTERVAL BETWEEN INOCULATION AND TREATMENT	RESULT IN 20 HRS.	FINAL RESULT *
		Broth (cc.)	Bacterio-phage (cc)			
1	50 m.	0 5		Simultan.	D	D
2	50 m.		0 5	Simultan.	L&W	S
3	50 m.		0 5	Simultan.	L&W	S
4	50 m.	0 5		15 min	D	D
5	50 m.		0.5	15 min.	Sl sick	S
6	50 m.		0 5	15 min	Sl. sick	S
7	50 m.	0 5		30 min.	D	D
8	50 m.		0 5	30 min.	Sl. sick	S
9	50 m		0.5	30 min	Sl. sick	S
10	50 m	0.5		1 hour	D	D
11	50 m.		0 5	1 hour	Sick	S
12	50 m.		0 5	1 hour	Sl. sick	S
13	50 m.	0.5		2 hours	D	D
14	50 m.		0.5	2 hours	Sl sick	S
15	50 m.		0 5	2 hours	Sl. sick	S
16	50 m.	0 5		3 hours	D	D
17	50 m		0 5	3 hours	Sl sick	S
18	50 m.		0.5	3 hours	Sick	S
19	50 m	0 5		3½ hours	D	D
20	50 m.		0 5	3½ hours	Sick	S
21	50 m.		0 5	3½ hours	Active	S
22	50 m	0.5		4 hours	D	D
23	50 m		0 5	4 hours	D	D
24	50 m		0 5	4 hours	Sl. sick	S
25	50 m.		0 5	4½ hours	D	D
26	50 m.		0 5	4½ hours	D	D
27	50 m.			4½ hours	D	D
28	10 m.				D	D
29	2 m.				D	D
30	1 m.				Sl. sick	S
31			2		L&W	S
32			4		L&W	S

* Control mice began to die in 5 hours and at least half of them were dead before 7 hours after inoculation.

S—survived D—died. L&W—living and well.

products rather than to the phage and occurs following much smaller doses of coliphage than staphylococcal phage, because of differences, as Northrop has suggested,[48] in the nature of the proteins of these two organisms. Although this protein shock seldom does harm, because the temperature promptly falls again, it is to be avoided because of the discomfort of the patient. In our experience it is not a necessary part of the successful therapeutic use of bacteriophage because we have frequently seen the septicemia controlled and the urine cleared of organisms without this reaction.

Organisms other than *E. coli* frequently found in cystitis cases are *Bacillus proteus*, *Pseudomonas pyocyanea*, nonhemolytic streptococci, and nonhemolytic staphylococci. They are frequently associated with *E. coli* but on the other hand may be the only organisms involved. The only one of these organisms that we have found susceptible to bacteriophage is *Pseudomonas pyocyanea*, and many strains of this organism are resistant. When the phage is 'doubly potent,' as we shall explain below, infections due to this organism will respond in a satisfactory manner to phage therapy.

C. PHAGE THERAPY IN STAPHYLOCOCCAL INFECTIONS

Our chief experience in phage therapy has been the treatment of local and generalized infection due to staphylococci. This work began in 1931. The bacteriophage used at the beginning was prepared periodically in d'Herelle's laboratory in New Haven and was sent to us in batches as needed. It was used at first on local staphylococcal infections seen in the out-patient department of the Presbyterian Hospital.

1. *Laboratory Observations and Experiments*

The first two batches were apparently successful in shortening the duration of most of the lesions treated, and lysis of the strains in the test tube occurred regularly. However, on the arrival of the third batch the results suddenly became poor, and simultaneously the phage failed with many strains to produce complete lysis in the test tube. When we reported this to d'Herelle and Rakieten it was said that in some way or other this phage must have lost its potency during the final heating process (60° C. for one hour on three successive days). The use of one of the next batches, however, met with varying success. On inquiring whether in the method of preparation of the different batches of phages there were any changes that might have affected the potency, it was learned that in the last one the phage was propagated at the expense of only one strain of staphylococcus, whereas for the preparation of phage of the first and second batches, ten to twelve strains of staphylococci were used.

The same failure occurred when we produced our own phage by the method used by MacNeal,[48] who supplied us with five different strains of polyvalent *staphylococcus* phage and a stock culture upon which he propagated them. The five phages were propagated separately in plain broth at a pH of 7.6 to 7.8, using 0.1 cc. of the phage to 10 cc. of broth. The tubes were incubated at 37° C. overnight and, if found to be clear, they were filtered and pooled before being used in the clinic.

The results at the beginning were encouraging. However, after a short period of success, we were suddenly faced with a series of failures. Simultaneously, it was found that the bacteriophage was beginning to lose its power to produce complete lysis *in vitro*. The phage was then tested against old strains that it had formerly lysed, and it was found to be impotent.

Not recognizing the cause of this deterioration, we made an immediate shift to strain BL obtained from Gratia then of the University of Brussels. This was found to be effective not only in the laboratory but in the clinic. Its period of success lasted for about two months, only to be followed by a series of failures. This time, however, the laboratory tests still showed a complete visible lysis of most of the strains of staphylococci, even to the eighth decimal dilution of phage, which d'Herelle considered a high degree of potency. No explanation for it could be offered either by d'Herelle, MacNeal, or Gratia, to whom we appealed for help. Greatly discouraged, we were just about ready to give up the whole study, when the doctors in charge of a doctor's wife with a desperate case of staphylococcal septicemia following a carbuncle of the face, when everything else had failed, called for a potent phage. We had none that we could depend upon, so we called upon Dr. MacNeal. He responded, and by a series of intravenous injections, begun when the patient was struggling for breath and closure of the glottis was threatened by the edema, he dramatically turned the tide in the patient's favor. The blood became sterile and she made a prompt and complete recovery. We then tested the potency of MacNeal's phage against the causative organism and found that his had a titer 10 to 100 times as strong as any of our own.

It was obvious that the potency of the bacteriophages we were using was not constant or adequate, and we determined to find some method of improving and insuring the potency of the phage. At that time, no standard method of phage preparation had been recognized, although many methods had been proposed. We decided to investigate certain of the factors that might be involved, for example, the media employed, the bacterial strain used for propagating the phage, the concentration of the bacterial inoculum, the amount of phage used, the optimum temperature, and the method of testing for potency.

A. CULTURE MEDIUM

For a propagating medium, some authors have recommended plain meat-infusion broth; but MacNeal has advised asparagin for intravenous injection, to minimize the protein content, whereas others, including d'Herelle and his followers, favor 'savita' medium. This is made out of 1 per cent 'savita' extract, 0.5 per cent of sodium chloride, and adjusted to a pH of 8.1, which after being boiled and autoclaved becomes 7.5 to 7.8. We have employed all three in this study. We have not used the papain-digest broth recommended by Asheshov. We found that asparagin was not sufficiently nutritive for the development of either bacteria or bacteriophage. Staphylococci grew in it very slowly, yielding about 500 to 750 million organisms per cubic centimeter after 24 hours of incubation at 37°, and still less growth at a temperature of 32°, or lower. Asparagin was, therefore, abandoned and replaced by 'savita' medium.

A comparative study of staph-phage preparations in meat-infusion broth and 'savita' medium showed that the lysis of bacteria in broth is accomplished in a shorter period of time than in 'savita' On the other hand, in our clinical application of bacteriophage for the treatment of staphylococcus infections, such as furuncles and carbuncles, in which bacteriophage was injected directly into the lesion, we found that broth phage occasionally gave a severe local reaction, whereas this never occurred with 'savita' phage. Furthermore, when injected subcutaneously, 'savita' phage gave practically no reaction, whereas the area injected with broth phage was sometimes surrounded by a large zone of redness.

B. THE OPTIMUM TEMPERATURE FOR PROPAGATION

There has been no consensus with regard to the optimum temperature for the propagation of phage. In the bacteriophage laboratory of d'Herelle in Paris, the phages are prepared at 33° C., the same temperature used by MacNeal, of New York.[50] Gratia[51] at Liége prepares his phages at 37° C., and Larkum[52] of Lansing, Michigan, and Asheshov[53] of India prefer the temperature of 22° C. A study of this question in our laboratory has shown that a temperature of 32° to 33° C. was the most favorable for our staphylococcus phages. The experiments showed that phages prepared at 32° C. remained clear indefinitely, while bacteriophages made under identical conditions but at a temperature of 37° C. developed secondary growth after standing for one or two weeks at room temperature. No significant difference in potency as measured by plaque count and lytic action in broth was found between phages prepared at room temperature and at 32° C. However, preparation at room temperature was abandoned after it was found that some strains of staphylococci, especially those just isolated from the body, grew at room temperature

very slowly so that the control tubes were often found to be still clear after twenty-four hours. This might be a source of error. Besides, even a weak bacteriophage will often produce a complete visible lysis at room temperature. Thus, a weakening of phage can be more easily missed at room temperature than at 32° C. incubation.

C. BACTERIAL CULTURES

Some laboratories use a single strain of staphylococcus for the preparation of a number of staph-phages. Others employ a specific strain for each bacteriophage. Still others propagate their phages against a mixture of bacterial cultures after adding some freshly isolated strains each time that phage is made. We found that any bacteriophage propagated for a long time in contact with a single strain begins to lose its potency with respect to other strains, but if propagated on a mixture of active strains or if propagated separately on 10 to 12 active strains and if the resulting phages are pooled, the combined phages will maintain not only a high titer but a broad polyvalency against cultures from fresh infections. We repeatedly found that when a phage began to lose its potency, it could be restored by propagating it against other suitable strains of staphylococci—'suitable strains' being those on which the phage corpuscles rapidly multiplied.

D. TESTING THE POTENCY OF BACTERIOPHAGE

Our difficulties in maintaining the potency of phage, both in the laboratory and in the clinic, induced us to study all the various methods proposed for testing potency. The method most commonly used for testing the potency of bacteriophage against any given culture was to bring the bacteriophage into contact with a thin suspension of the bacteria in broth, with a control. After incubation, for a variable length of time the clarity of the phage tube was compared with the control. Any difference indicated phage activity. If the phage tube was perfectly clear, it was considered a potent phage. Relative clarity with the control might be designated 1, 2, 3, or 4 plus.

In 1926, d'Herelle [2] preferred to determine the activity of bacteriophage by the actual count of 'corpuscles.' According to Bulgakov,[51] of d'Herelle's laboratory in Paris, phage that yields a number of plaques when diluted 10^{-6} is considered to be potent. Others measure the potency of bacteriophage by a method of successive dilutions in liquid medium proposed by Appelmans.[53] This author considers that the higher the dilution of phage in which complete lysis of bacteria is obtained, the better the bacteriophage. Sertic and Bulgakov [54] were in accord with d'Herelle's idea that the potency of phage is proportional to the rapidity with which

phage corpuscles multiply. The fact that many methods have been devised for determining the potency of bacteriophage indicates that none has been found to be entirely satisfactory. Furthermore, any method for determining potency *in vitro must be correlated with clinical results* if the laboratory is going to be of direct service in obtainng practical results in phage therapy.

When a phage begins to yield poor results in the clinic, it usually gives some indication of its loss of potency in the laboratory tests, for example, by failing to produce complete lysis first of heterologous strains and then of the homologous strain upon which it had been propagated. One of our phages (BL)—from Gratia—however, began to lose its potency clinically without any diminution of its lytic power in broth. But, when a loopful of the cleared culture, without previous filtration, was plated on blood agar, we found to our surprise that the culture gave a profuse growth of individual nonconfluent colonies on the plate. The colonies were yellowish and did not differ in any respect from normal staphylococcus colonies. When these colonies were smeared on slides and stained, the individual organisms appeared normal. When transplanted into broth and incubated, they gave a normal turbidity, and after being brought into contact with the same bacteriophage, they underwent complete visible lysis again. However, when this cleared culture was again plated on blood agar, it yielded typical staphylococcus colonies again. Some of the cleared tubes yielding growth were found to remain clear for a month while standing at room temperature, and during this period, each time they were plated they gave an extensive growth of separated, normal-looking colonies of staphylococci. When plain agar plates were inoculated instead of blood agar, growth was considerably less abundant and in some cases entirely absent (see Fig. 6). It was evident that blood agar was a better medium for the 'invisible' staphylococci, or else the blood contained some element that inhibited the phage. However, the coincidence of this phenomenon with the clinical evidence of lack of potency suggested a re-examination by the same method of the clinically more potent phages.

A comparison of several different phages showed that some produced complete lysis of all of the bacteria, and repeated plating failed to show growth; whereas others cleared the cultures, but yielded growth of normal or abnormal colonies when the cleared tubes were plated. Still others failed to clear the bacterial suspensions at all. These three groups, we believe, represent three different degrees of potency, and yet they cannot be correlated exactly with other measures of phage potency. Bacteriophage may produce complete visible lysis of a large percentage of bacterial strains, and it may be of a high titer, as shown by titration through decimal dilutions in liquid medium or by any method of enu-

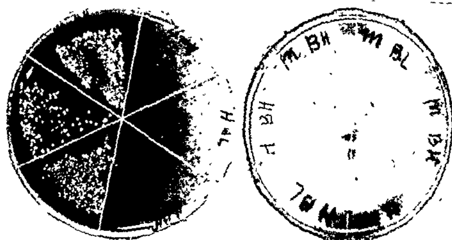


FIG 6 If the bacteriophage is not 'doubly potent,' blood agar (left) will yield growth from the same cleared cultures which fail to grow when transplanted on plain agar (right).

meration of the bacteriophage corpuscles; still, if the phage is not really potent, cleared bacterial suspensions will yield growth of normal or abnormal colonies when plated on blood agar. This seems to be the first evidence of a fall in potency, for it is soon followed by a diminishing ability to produce complete visible lysis of bacteria in the test tube and by a drop in the number of bacteriophage corpuscles and a slowing of the rate of their multiplication. For this reason, this simple method of testing phage potency is of practical significance and might be profitably employed whenever any clinical use is to be made of the phage.

E. THE DOSAGE OF BACTERIA FOR THE PROPAGATION OF BACTERIOPHAGE

In 1926, d'Herelle[2] recommended a heavy inoculum of bacteria approximating 250 M/cc. of medium, but a number of workers prefer a much smaller amount. MacNeal,[57] for example, in 1931, prepared his phages in bacterial suspensions estimated to contain 25 to 50 M/cc. To correlate these theories with the question of potency as indicated by absence of growth of the cleared culture on blood-agar plates, the following experiment was set up. A series of 10 tubes containing 10 cc. of plain broth was used (Table II). The amount of inoculation ranged as follows: 25, 75, 150, 225, and 300 M/cc. of organisms, 2 tubes being used for each dose of organisms; 0.1 cc. of phage containing approximately 10 million 'corpuscles' was added. The tubes were then incubated at 32° C. for twenty-four hours. When examined, it was found that the tubes inoculated with 25, 75, and 150 M/cc. of bacteria were clear, while those prepared at the expense of 225 M/cc. and 300 M/cc. were translucent. A drop from each tube was then plated on blood-agar plates and

incubated overnight. When the plates were examined on the following day, the parts of the plate inoculated with one drop from the tubes receiving the smallest dose of organisms yielded a very rich growth of separate colonies. The colonies were considerably less abundant from the tube inoculated with 75 M/cc. No colonies grew out of the tubes inoculated with 150 M/cc., 225 M/cc., and 300 M/cc. of bacteria. (See Table II.)

TABLE II

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF B-59-A PHAGE INOCULATED	APPEARANCE OF BROTH AFTER 24 HOURS	GROWTH ON THE BLOOD AGAR	APPEARANCE OF BROTH			TITER OF PHAGE AS TESTED BY PLAQUE COUNT
				72 hr	96 hr	2 wk	
25 million	0.1 cc. = 10 million corpuscles	Clear	Abundant	Slightly cloudy	Cloudy	Cloudy	10^{-6}
75 million	0.1 cc = 10 million corpuscles	Clear	Moderate	Clear	*Slightly cloudy*	*Cloudy*	*Not tested*
150 million	0 1 cc. = 10 million corpuscles	Clear	Absent	Clear	Clear	Clear	10^{-8}
225 million	0.1 cc. = 10 million corpuscles	Translucent	Absent	Clear	*Clear*	*Clear*	*Not tested*
300 million	0.1 cc. = 10 million corpuscles	Translucent	Absent	Clear	Clear	Clear	10^{-7}

The phages prepared at the expense of 25 M/cc., 150 M/cc., and 300 M/cc. were tested for their potency by the plaque count. Plaques appeared in the eighth decimal dilution of the phage prepared at the expense of 150 M/cc., while the limit of the phage prepared with the 300 M/cc. inoculum was the seventh dilution, and the limit of the phage prepared with the 25 M/cc. inoculum was the sixth dilution. These experiments were repeated a number of times and according to the plaque-count method of testing potency, the optimum inoculum was found to be from 150 M/cc. to 250 M/cc. of organisms. All of these experiments seem to indicate the following facts:

1. 'Savita' medium is superior to plain meat-infusion broth chiefly because of the relative *infrequency* of uncomfortable reactions in the patient, but laboratory tests indicate that the broth phages are more potent

2 The optimum temperature for the propagation of staphylococcus bacteriophage seems to be 32° or 33° C.

3. Although the potency of phage may be maintained for several months by its propagation at the expense of a single strain of bacteria, it may sooner or later lose its potency thereby, and have to be restored by propagation against another suitable strain or a mixture of strains.

4. More potent phages are obtained by using large inoculations of bacteria (150 M/cc. to 250 M/cc.) and small inoculations of phage 0 1 to 0 01 cc

(approximately 1 to 10 million corpuscles) in 10 cc. of medium, rather than with smaller quantities of bacteria and larger amounts of phage.

5. The earliest indication of loss of potency is the appearance of growth on blood agar from the cleared bacterial suspension. This is followed by a failure to produce lysis and a slowing of the rate of multiplication of phage corpuscles. If the bacteriophage not only clears the culture but prevents the growth of organisms from the cleared culture when a drop of it is transferred to a blood-agar plate, it is called 'doubly potent.' The test should be used on the strains of all cases receiving phage treatment either local or general. If 'double potent' phage is used, we are confident that a high rate of success will be achieved

2. *Clinical Observation and Experiences*

The importance of the double potency test was amply demonstrated by our clinical use of bacteriophage both in localized and generalized staphylococcus infections. We seldom encountered the failures that were all too frequent and obvious in the earlier years of our experience, that is, prior to 1936. This is clearly indicated in the tabulation of our results in staphylococcus septicemia reported in 1940,[13] divided into the cases treated with bacteriophage prior to October 1936 and those treated between that date and October 1938. (See Table III.) It is true that larger doses of phage were used in the latter period, but that was partly because more of the patients lived longer and we emphasized giving as large doses as could be tolerated.

TABLE III

SUMMARY OF FINAL RESULTS IN CASES OF STAPHYLOCOCCUS SEPTICEMIA

TIME OF TREATMENT	NO OF CASES	NO. OF SURVIVALS	NO OF DEATHS	MORTALITY PER CENT
Before Oct. 1936	15	4	11	73.3
Oct. 1936 to Oct. 1938	21	15	6	28.5
Total	36	19	17	47.2
Controls	54	10	44	81.4

When the cases were divided according to the double-potency test, it is seen that the mortality among those receiving the phage of known potency was only 23.8 per cent.

We are convinced that the double-potency test indicates the difference between bacteriostatic and bactericidal activity or between partial and complete death of the organisms *in vitro*. That does not mean that *in vivo* all of the bacteria are destroyed by the phage in cases so

TABLE IV

RESULTS OF POTENCY TESTS

LYSIS IN SAVITA	GROWTH ON PLATE 5 PER CENT SHEEP'S BLOOD	NO. OF PATIENTS	NO. OF DEATHS	NO. OF SURVIVALS	MORTALITY PER CENT
4+	no growth	21	5	16	23 8 *
4+	1+ to 3+ growth	5	4	1	80
4+	not plated	8	6	2	75
2+	.	1	1		100
0	..	1	1		100
		36	17	19	47 2

4+ = complete lysis. 2+ = partial lysis. 0 = no lysis

* If there is complete, or a 4+ lysis, in the tube and no growth lysis on the plate, it is called 'double-potency' phage.

treated, but it is reasonable to suppose that with the more potent phage the body has a better chance of controlling the infection Krueger[7] has expressed his doubt that bacteriophage *ever* destroys bacteria in the body, but our experiments indicate the possibility if not the probability of such action, and these findings are supported by the evidence brought out by Dubos and Straus.[47] It seems probable that in the majority of cases of staphylococcus septicemia that are successfully treated with bacteriophage, a phage has been used that is doubly potent against the organism, whereas in the majority of the failures the organisms are resistant or only partially susceptible to the phage. Divergent results of phage therapy by different observers may well be explained on this basis.

The following successful cases are reported as examples of patients in whom the bacteriophage played an important, if not an essential role in recovery. These six cases are selected also because they illustrate different phases of this disease:

Case I L.B., aged 29 years, female, was admitted to the gynecological service of Sloane Hospital for a myomectomy, which was performed on 29 September 1936. After operation she ran a stormy course with a temperature spiking between 101° and 105°. This was associated with some swelling and redness of her wound. On 1 October, she had a shaking chill. Blood cultures taken during chill revealed hemolytic *Staphylococcus aureus*, as did a culture taken at the lower angle of her wound. The wound was then spread slightly at its upper and lower poles, and through-and-through drains were placed. In spite of transfusions and the usual supportive treatment, her course was progres-

sively downhill over a period of twenty days, during the last three of which she was irrational and at times semicomatose, and she was expected to die. Five blood cultures were taken during this period and all were positive. On 20 October she was given 50 cc. of bacteriophage intravenously in one dose. A blood culture taken just before starting phage was positive. Twenty-four hours later, another culture, taken just before the second dose of 50 cc. of phage, was negative. All cultures subsequent to this date were negative. In spite of a marked improvement in her general condition, she continued to run a temperature daily between normal and 101°. She was therefore given an additional 50 cc. of phage on 26 October. Examination then revealed a pelvic abscess, which later ruptured spontaneously into the wound. Following this the course was uneventful, and the patient was discharged completely healed. The wound was dressed with daily irrigation and instillations of bacteriophage.

This is a brilliant result in a postoperative infection with subsequent sepsis. The patient's life was despaired of before the phage was started. The immediate disappearance of organisms from the blood stream after the first dose was dramatic, but hardly less so than the patient's clinical response once her sepsis was under control. It illustrates that the phage may be given in large initial amounts to patients who are critically ill

Case II: S.D., aged 35 years, Negro, male, was admitted to the surgical wards of the Presbyterian Hospital on 14 March 1937, with a diagnosis of prolapsed and ulcerating hemorrhoids. Nine days prior to admission he began to have pain in his rectum, and seven days before admission he had a shaking chill. On admission his temperature was 102°. Examination showed large protruding ulcerated hemorrhoids. Blood cultures on 16 and 18 March revealed hemolytic *Staphylococcus aureus*. Rectal examination showed a large edematous and indurated seminal vesicle on the right. He was transferred to the genitourinary service and the seminal vesicle was opened and drained through a perineal incision. The operation was thought to establish inadequate drainage. Because of the persistence of the positive blood culture, bacteriophage was started on 19 March, intravenously and also applied locally. He showed immediate improvement although the blood cultures remained positive for sixteen days after starting phage The patient made an uneventful recovery and was discharged. Subsequently he was readmitted on two occasions for drainage of two Brodie abscesses of his right tibia and later for similar lesions in his left femur and left tibia. These also revealed hemolytic *Staphylococcus aureus* and probably represent delayed appearance of metastatic abscesses. They were treated locally with bacteriophage and promptly healed.

This is an interesting case of staphylococcus septicemia. The distributing focus on admission was the right seminal vesicle. He had his first symptom of sepsis seven days prior to admission and thirteen days before receiving bacteriophage. In view of the long duration of his

sepsis, it was surprising that he did not develop demonstrable foci in some of his viscera. The second blood culture before phage was given revealed the presence of a spontaneous bacteriophage, which may have inhibited the growth of organisms in his blood and minimized the establishment of metastatic foci. It did not, however, prevent the growth of organisms when his blood was transferred to artificial media. The phenomenon of the spontaneous appearance of bacteriophage we have observed in one other case.

Case III: E.A., aged 54, male, was admitted to a suburban hospital 5 April 1937 for an alveolar abscess. Two weeks before admission, he began to have pain and swelling in his nose. The involved area was drained, but he continued to have fever, headaches, and generalized body pains. Temperature on admission was 102° to 104°. The mucous membranes covering hard palate and gums were swollen and red, and pus was draining from the upper gum margin. Blood culture on admission revealed hemolytic *Staphylococcus aureus*. He was given one dose of prontylin. It was discontinued, and over the next three days he received alcohol 10 per cent infusions. Bacteriophage was started on the third day. At this time he had evidence of fluid in his right chest. This was tapped and a yellow cloudy fluid was withdrawn, which yielded the organism. Ten cubic centimeters of phage were introduced into the pleural cavity. During the next 20 days, he received 16 intravenous injections of 50 cc. of bacteriophage. Bacteriophage was also applied locally to the gum area. No further intrapleural injections were necessary. The last positive blood culture was obtained on the ninth day, or 5 days after the first dose of phage. The patient then proceeded to make an uneventful recovery.

This case illustrates a staphylococcus septicemia in a patient very ill with metastatic lesions in the lung and pleura. The fluid aspirated on the third day was the only fluid obtained from the chest. There was no evidence of a reaccumulation. This is another example of a recovery from a case of hemolytic *Staphylococcus aureus* sepsis in a patient who was desperately ill at the time bacteriophage was started.

Case IV: F.P., aged 8 months, male, was admitted to Babies Hospital, 1 June 1937, with a diagnosis of acute osteomyelitis of right tibia and cellulitis of the great right toe. Ten days prior to admission he had contracted an upper respiratory infection. This was followed by a cellulitis with threatening gangrene of his toe. Temperature on admission was 105°. Blood culture on 2 June revealed hemolytic *Staphylococcus aureus*. Intravenous bacteriophage was started on the second day after the first positive blood culture During the course of the next 20 days, he received a total of 125 cc of bacteriophage intravenously. Blood cultures on the 13th and 18th days were positive. Subsequent blood cultures were all negative. Intravenous bacteriophage was continued for 6 days after his last positive blood culture. During the course of phage therapy, the osteomyelitis in his right tibia was incised and drained on

TABLE V

THE EFFECT OF STAPHYLOCOCCUS BACTERIOPHAGE ALONE AND IN COMBINATION WITH CEEPRYN AND PHEMEROL AS COMPARED WITH PENICILLIN ON THE CONTROL OF EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN EMBRYONATED EGGS

SUBSTANCES INOCULATED	NUMBER OF EGGS	DEATH IN 24 HOURS	CULTURES		DEAD 2-4 DAYS	CULTURE		DEAD 5-7 DAYS	CULTURE		LIVED 7 DAYS OR MORE	CULTURE		% OF EGGS SURVIVED 7 DAYS OR MORE	% OF EGGS WHICH WERE FREE OF GROWTH
			Sterile	Growth		Sterile	Growth		Sterile	Growth		Sterile	Growth		
Culture alone	32	30	—	30	2	—	2	—	—	—	—	—	—	—	—
Culture + phage	19	—	—	—	7	—	7	6	1	5	6	1	5	31	10.5
Culture + phage + ceepryn	17	—	—	—	6	1	5	5	1	4	6	—	6	35	11.7
Culture + ceepryn	4	2	—	2	2	—	2	—	—	—	—	—	—	—	—
Culture + phage + phemerol	15	2	—	2	4	1	3	3	—	3	6	1	5	40	13.3
Culture + phemerol	4	1	—	1	3	—	3	—	—	—	—	—	—	—	—
Culture + penicillin	50	2	1	1	2	1	1	14	10	4	32	29	3	64	82

of the chorioallantoic membrane, avoiding the larger vessels. This was done through a small hole made with an egg piercer. In order to relieve pressure within the egg and to allow a free passage of fluid, another small hole was drilled into the air sac. After the inoculation, both holes were sealed with surgical Scotch tape. The inoculated eggs were kept in the incubators at 38.5 to 39.5° C. The criteria of effectiveness were not only the survival of the eggs but the sterilization of the bacteria in the eggs during the seven-day period of observation. The results are shown in Table v.

To summarize these results, we may say that:

1. The inoculation of the chorioallantoic fluid of 10-day-old chick embryos with 0.1 cc. of a savita broth culture of *Staphylococcus aureus* consistently brought about the death of the embryos within 24 hours.

2. The administration of staphylococcus bacteriophage immediately following the inoculation of bacteria prolonged the life of all chick embryos.

3 The cultures of the egg material of the phage-treated embryonated eggs resulted in a decrease and a degeneration of bacterial growth. However, a complete sterilization was seldom achieved.

4. The presence of bacteriophage in the chorioallantoic fluid usually did not prevent the bacterial invasion of the blood stream of the embryos, although the appearance of phage in the blood usually could be demonstrated.

5. Active multiplication of bacteriophage occurred in the majority of the cases when the undiluted or lower dilutions of phage were used. But dilutions of 10^{-5} or higher seldom multiplied and often completely disappeared.

6. The *in vitro* experiments carried out with chorioallantoic fluid and with a mixture of the egg material showed that staphylococcus phage not only multiplied in these fluids but also produced a lysis of susceptible bacteria suspended in them The presence of embryonic blood did not prevent bacterial lysis, although there was some inhibition.

7. The addition of ceepryn or phemerol did not significantly change the therapeutic results of bacteriophage treatment of the infected embryonated eggs.

8. The administration of 100 units of the calcium salt of penicillin any time within 6 hours following the bacterial inoculation resulted in survival of the majority of the embryonated eggs for the 7-day experimental period and in hatching of eggs when incubation was prolonged beyond this period.

9. The therapeutic results were essentially the same when 10 units of penicillin was administered but survivals fell off when 1 unit was used.

10. The sterilization of the infected eggs, however, depended upon the numbers of units of penicillin used. A complete sterilization occurred in 76 per cent of all eggs inoculated with 100 units of penicillin.

11. The administration of 28 5 or 57 mg. of sulfathiazole simultaneously with the bacteria failed to prolong the life of the embryonated eggs or to produce significant bacterial inhibition of the test dose.

12. Antistaphylococcus rabbit serum (Julianelle) and staphylococcus anti-

toxin horse serum in 0.1 and 0.2 cc. amounts produced some prolongation of the life of the eggs, but the antibacterial effect of these substances was insignificant.

13. Carboxymethoxylamine (CMOA) tested in 0.5 per cent concentration produced a sterilizing effect *in vivo* and *in vitro* in a majority of the cases, but was found toxic for the embryos. Only the local use of this drug can be considered safe.

14. Vioform in 0.5 per cent concentration was not toxic but possessed only a slight bacteriostatic effect.

15. The new antibiotic agent, Bacitracin, produced by the Treacy strain of *Bacillus subtilis* discovered in our laboratory, exerted a significant protective effect of the embryos, but in the concentrations used, it was not as efficient as penicillin.

B. CLINICAL COMPARISONS OF BACTERIOPHAGE AND PENICILLIN

The results of penicillin treatment both in local and general staphylococcal infection are given in Chapter XXII. In a large series of cases of septicemia, the mortality is still about 25 per cent, the figure obtained in our small series of septicemia cases treated with bacteriophage from 1936 to 1938. We have seen and obtained the same dramatic results with both methods of therapy including cases of osteomyelitis with septicemia in which a breakdown of the bone was avoided. But these results are obtained more consistently with penicillin, and we have never controlled infection in a suppurative arthritis with bacteriophage. The experimental results quoted above may very well represent the relative merits of the two treatments in their clinical application. In cutaneous and subcutaneous staphylococcal infections, penicillin also yields a consistently higher average of successful results although with doubly potent bacteriophage the same dramatic responses have often been seen. The synergism of these two aspects is worthy of study.[62]

However, the necessity for close collaboration with the laboratory makes the general availability of potent bacteriophage impossible. Unless and until some means are found to produce commercially a bacteriophage that will have as wide a coverage of staphylococcal strains as penicillin, be potent enough to kill the organisms, and have that potency preserved over a long period of time, it cannot compete with penicillin. The effort to find ways and means to produce such a phage should be continued because bacteriophage has one theoretical advantage over penicillin, the power to propagate itself at the site of the infection. This may very well be nullified by the inhibiting action of various body fluids, pus and blood, which do not inhibit penicillin, and if repeated or continuous application of the active principle will do as well as propagation, this property may be of minor importance. For the present at least, the weight of evidence lies in favor of penicillin.

and its general availability and use has already had a striking effect on reducing the incidence of staphylococcal septicemia, a result not accomplished by the sulfonamides with staphylococcal infections, but achieved by them with respect to hemolytic streptococcal septicemia.

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Research Council. Through the latter, it was distributed to study units and individuals who were directed to make careful observations and reports on the conditions of its use and the results obtained. A preliminary report of 500 accumulated cases was made by the committee in 1943.[6] Production steadily increased and it became possible to supply the armed forces with their full requirements and have enough available for suitable civilian cases. One of the earliest reports on soldiers returning from the front with infected compound fractures was made by Lyons.[7] A group of established surgical infections in civilians was reported by the study units.[8] The high cost of production and the limited supply at first prevented the indiscriminate use of penicillin. The results of its prophylactic and therapeutic use in surgical infections of all kinds had to be scrutinized and analyzed very carefully in a large number of cases before its proper appraisal could be determined.

The development of penicillin has opened up a wide field of research in bacterial diseases, and visions of wide application of the principle of bacterial antagonism have been given encouragement. Hundreds of workers have hopefully entered this field, and thousands of micro-organisms among bacterial groups and fungi have been studied for their antibacterial and antiviral activity. Past experience cautions against a too roseate hope of success, but persistent effort will bring progress step by step. The chief handicap to progress in this field lies in the fact that most of the so-called antibiotic substances are toxic. Thus, gramicidin isolated by Dubos[9] from *Bacillus brevis*, a soil organism, while it is active against many of the Gram-positive organisms, is toxic for the host in experimental animals. This limits its usefulness to local application in small quantities. A few of these substances, however, are nontoxic, and these should be pursued with all possible energy. Although streptomycin has met with striking success in a somewhat limited field, further search should be made for antibacterial agents against the Gram-negative rods, which are so persistent in war wounds, civilian accidental wounds, burns, and other chronic infections. Whether successful control of these organisms will lie in the field of chemotherapy or antibiotic therapy or some other therapy, time alone will tell.

I. PENICILLIN

A. PHARMACOLOGY AND TOXICOLOGY

When injected into the animal body, there is no indication that penicillin has any effect upon any of the normal functions of the various organs or tissues. Very little is known of its bacteriostatic action within the body. In the test tube, it has the power to prevent the multiplication

of susceptible bacteria, and, in adequate concentration, to destroy them. In sublethal doses, the bacteria grow large but do not divide. Some vital and essential function of the bacterial cell that it does not have in common with body cells is interfered with. Penicillin is readily absorbed from the site of injection and it permeates most of the tissues of the body. It is absorbed from the duodenum but is rapidly destroyed by the acid reaction of the stomach if taken by mouth unless an alkaline reaction can be maintained. If given intravenously, it is rapidly eliminated by the kidneys and most of it passes through unchanged. It can be recovered from the urine and used again.[10] That which cannot be recovered disappears somewhere in the body. Its presence can be determined in the blood by standard tests on staphylococcal or streptococcal cultures or other test organisms. Its action is not inhibited by blood, pus, split-protein products, or para-aminobenzoic acid, as are the sulfonamides.[11] Its therapeutic action depends upon the maintenance of an effective blood level in systemic infections or on adequate concentration locally for localized infections.

If administered intramuscularly or intravenously, it does not readily enter the spinal fluid. Infections of the meninges therefore must be treated by the intrathecal route.[12] Efforts have been made to find a medium in which it could be injected once a day to act as a depot of slow absorption. A number of substances have been suggested. The ideal medium will be one that is slowly but completely absorbed. A daily injection in a beeswax and peanut-oil medium has been successfully employed clinically,[13] but absorption from this medium varies in different individuals and cannot be depended upon to give adequate blood levels unless administered in very large doses Sensitivity to other ingredients of the medium, particularly pollens and other proteins, is a real danger of this method of treatment. Sterile abscesses frequently form.

In therapeutic doses, penicillin does not injure leukocytes or interfere with tissue culture of connective tissue, or epithelium.[14] There is no indication of toxicity with doses as high as 20 million units a day and, when applied to local surfaces in concentrations as high as 10,000 units per cc. there is no evidence of local injury to tissue. Local application to an infected surface usually results in a decreased exudate, but whether this is due to bacterial inhibition or leukocyte repulsion is not certain. If applied to a wound surface as a wet dressing, it is rapidly absorbed, but if applied in a carbowax base, active quantities will still be present after 24 hours.

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B. Dosage

Just what an 'adequate' dose is, for systemic streptococcal or staphylococcal infections, has not been determined and probably differs with each case, depending on the number and virulence of the organisms and the rapidity of their dissemination through the body. Very sick septicemias due to *Staphylococcus aureus* have been brought under control by as little as 5000 units given intramuscularly every 3 hours, whereas others may need 5 or 10 times that amount. Whether introduced intravenously or intramuscularly in doses of 5,000 or 40,000 units, it is completely eliminated within 3 hours and it must be renewed.[10] In severe cases, probably every 2 hours is safer in order to maintain a continuous effect by intramuscular injection. Continuous action may also be obtained either by intravenous infusion or subcutaneous or intramuscular clysis. In the average case of moderately severe infection, a dose of 80,000 to 100,000 units a day divided into 8 doses at 3-hour intervals is generally effective. If there is no response in 48 hours, the dose should be doubled. If no benefit is evident in the next 24 hours, the dose should be doubled again. If no response is seen in one week it might as well be stopped.

C. The Limitations of Penicillin Therapy

1. *Bacteriological Limitations*

Of the limitations of penicillin therapy the bacteriological ones are the most important. The causative organism must be determined if possible, and tested against penicillin *in vitro*. In most instances, it will not be effective against the Gram-negative rods.[15] If they are present in the culture, mixed with susceptible organisms such as Gram-positive cocci, the latter may be selected out by the penicillin and be destroyed, or they may be protected by the penicillin-inhibiting action of the Gram-negative rods.[16] In any infection, therefore, a complete bacteriological analysis should be made and the individual species tested for susceptibility or inhibiting action on the penicillin. Penicillin is not as effective in mixed infections of individually susceptible organisms as in pure infections.[3] The cause of this has not been determined. Although in the test tube it seems to be as potent against the hemolytic streptococcus as against the staphylococcus, the clinical results are more favorable in the pure staphylococcal infections. This may be due to the fact that the hemolytic streptococcus infections that have called for penicillin have been those that have not yielded to one or several of the sulfonamides, thus effective treatment has often been delayed. With staphylococcal infections, the recognition of the indaequacy of the sulfonamides usually leads the doctor to call for penicillin earlier.

2. *Toxic Limitations*

The chief toxic manifestation so far observed has been an urticarial rash. This rash has sometimes come on promptly after the first administration of the penicillin, but more often after a week or two of treatment.[11] It is usually only mildly annoying and is not accompanied by any other toxic reactions. Occasionally, however, it is very severe, demanding cessation of the treatment. It generally clears up promptly when treatment is stopped but may linger for a few days. In some instances it has appeared a day or so after penicillin treatment has been stopped. In several cases of gas gangrene that have been given large doses of penicillin, the local infection has seemed to come under control, but the patients have died a toxic death. This is most likely due to toxemia from the infection rather than from penicillin, but the possibility of the latter must be kept in mind.

Most patients who have received big doses have tolerated them very well. There has been a recent tendency to give doses ranging from a half to one million units a day in order to have a wide enough margin above the adequate therapeutic level to avoid the development of drug fastness. Allergic manifestations seem to be on the increase.

3 *The Limitation of Time*

The duration of illness before the administration of penicillin is an important factor in the outcome of treatment, particularly if there has been necrosis of tissue. If there has been no necrosis and the organism is susceptible, one may expect a rapid resolution of the infection without the necessity for surgery. If, however, there has been necrosis of tissue, it must be removed surgically or spontaneously before resolution can take place. This is particularly true in acute hematogenous osteomyelitis. If penicillin is given within the first 2 or 3 days of the symptoms, a series of x-ray films of the bone may never reveal any breakdown of the architecture. If started on the fourth to the seventh or eighth day, the local symptoms may subside, but the x-ray series may show progressive destruction of the bone resulting either from the destructive action of the organisms or a shutting off of the blood supply. Gradually there may be evident a regeneration of this injured bone or there may be further destruction of the bone. Regeneration may take place after very extensive bone destruction, or, if the organisms have not all been destroyed, there may be a reactivity of the infection with abscess formation either in the bone or in the overlying soft parts or both. If the dose of penicillin is too small, the initiation of treatment too late, or the causative organism resistant, the course of the infection

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will more nearly approximate the natural course of the disease without drug treatment.

Such results as are seen with penicillin with great regularity in osteomyelitis are occasionally obtained by the sulfonamides especially when the hemolytic streptococcus is the causative agent and the patient is a very young child. With staphylococcal osteomyelitis similar results were obtained not infrequently with potent bacteriophage, but the superiority of penicillin over both of these other agents can hardly be questioned. This has been demonstrated not only clinically but experimentally.[18]

What has been said with respect to the element of time of penicillin administration in osteomyelitis in relation to the development of bone necrosis may also be applied to abscess formation in soft-part infections. In suppurative infections of the pleura or joints, the general administration of penicillin may bring them under control if it is given early, but if there is any delay, local application will be necessary, and in later stages, surgery may be required. It is therefore almost self-evident that the earlier the treatment can be instituted the better will be the results, particularly if it can be given before the breakdown of tissue.

4. *The Limitation of Drug Fastness*

Certain strains of bacteria belonging to generally susceptible groups, such as the staphylococci, are primarily resistant to penicillin and may be even antagonistic to it by producing penicillinase. Other strains primarily susceptible may become resistant during the course of treatment, if the dose is inadequate to destroy them. These resistant progeny may also produce penicillinase and thus nullify further treatment. Fleming feared that self-medication of penicillin by mouth might result in the development in any community of many resistant strains which would cause incurable infections in the future. Certain it is that as time goes on, more and more penicillin-resistant strains are found associated with human infection. This is an additional reason for using adequate dosage early in the treatment of any infection.

D. THE ANALYSIS AND SYNTHESIS OF PENICILLIN

Persistent efforts to analyze penicillin have been rewarded, and the chemical formula has been approximately if not exactly determined.[19] During the study it has been shown that commercial preparations are made up of varying proportions of five slightly different penicillins having some variation in their bacteriostatic action on the same susceptible organism. These have been designated penicillin F, G, K, X, and dihydro-F.[20]

Penicillin G is the most consistent performer, and recent commercial preparations have been made with the object of containing a very high

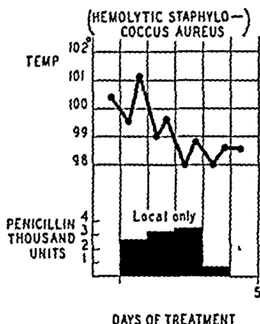


FIG. 1 A prompt subsidence of a large abscess of the face by local injection only. (Figs. 1-15 from Nelson's *Loose-Leaf Surgery* Courtesy of Thomas Nelson and Sons)

percentage of this fraction. Penicillin F and X are somewhat less active or less consistent in their action on the different susceptible species, and fraction K is either destroyed or eliminated so rapidly from the body that it cannot be depended upon for therapeutic results. It is thought that some of the unfavorable results of penicillin therapy in 1945 were due to a large proportion of penicillin K in some of the commercial preparations.

Synthesis of penicillin has been accomplished,[21] but this has not as yet been fully developed commercially, so that for the time being at least we shall have to depend upon the naturally produced antibiotic for adequate quantities of an inexpensive drug.

E. Illustrative Cases of Penicillin Treatment

The following cases show some of the successes and some of the failures of penicillin therapy, a fair cross section of the results generally obtained.

Case 1 J O. Age 18. No. 697813.

DIAGNOSIS. Abscess of the face and neck.

HISTORY. During the previous two months, the patient had had two large abscesses on the face, which had been incised and drained, leaving unsightly scars. Seven days before admission, an abscess began to form in the right lower cheek region, starting as a pimple about 3 cm. lateral to the angle of the mouth.

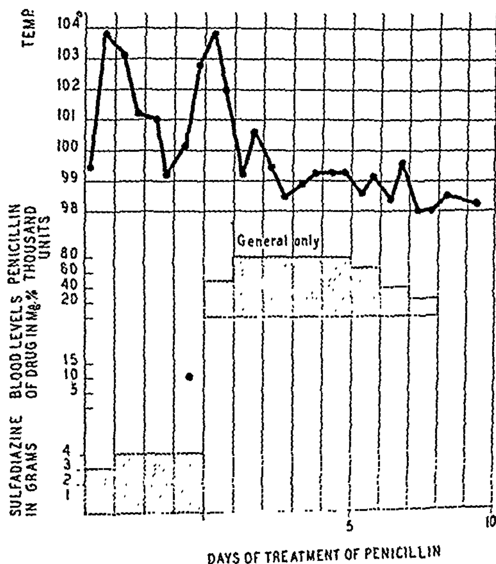


FIG. 2. A prompt response to penicillin when sulfadiazine had failed.

This steadily increased in size, with only moderate fever but marked malaise and pain beneath the tongue on swallowing and talking.

PHYSICAL EXAMINATION. The whole right cheek and the upper part of the neck were swollen and indurated with swelling in the floor of the mouth on the right side. In the center of the indurated area was a small opening discharging a small amount of pus.

COURSE. Cultures showed hemolytic *Staphylococcus aureus*. Approximately 3000 units of penicillin in 3 cc. were instilled each day into the abscess cavity

through the small opening in the cheek. Local heat was applied. There was a very rapid resolution of the process after only 4 days of treatment. (Fig. 1.)

This case illustrates the prompt resolution of a pure hemolytic staphylococcus infection following local application of penicillin without the necessity for surgery.

Case II: C.B. Age 32. No 726141.

DIAGNOSIS. Cellulitis of the cheek and orbit following osteomyelitis of the malar bone.

HISTORY. Two weeks before admission, a bilateral Caldwell-Luc operation had been performed. The right side quieted down after the operation, but the left side became painful and swollen There was no response to sulfathiazole or sulfadiazine, and the patient, who is a Mexican doctor, flew to New York for further treatment.

PHYSICAL EXAMINATION. There was marked swelling of the left cheek with closure of the left eye from edema of the eye lids and the orbit. His temperature was 99.6° F. but rapidly rose to 103.6° F. with headache and disorientation

COURSE. The patient was given sulfadiazine and his temperature fell during the next two days, but the local process gradually increased in severity. His temperature again mounted to 103.8° F. He was then given penicillin, 10,000 units every 3 hours intramuscularly, and soon after the second injection, he volunteered the information that he felt decidedly better. His temperature promptly fell to normal on the third day of treatment and remained there during the rest of his hospital stay. An x ray was taken just before he left the hospital, and this revealed an osteomyelitis of the malar bone. However, at that time, all the swelling of the cheek had completely subsided. Several months later, a sequestrum formed that had to be removed. (Fig. 2.)

This case illustrates the failure of sulfadiazine to control a *Staphylococcus aureus* infection of the face with osteomyelitis of the malar bone, but a prompt response to penicillin. The subsequent course proved, however, that the bone focus had not been completely sterilized, and surgical removal of the bone was required.

Case III: L. M. Age 53. No. 533080.

DIAGNOSIS Incipient lung abscess.

HISTORY. Six days before admission, 7 of the patient's upper teeth were removed because of abscesses. There was a moderate amount of pain, but no fever until the morning of the day of admission, when she suddenly developed a cough with a substernal chest pain and a severe shaking chill. She coughed up a teaspoonful of grayish pus and vomited from time to time during the day.

PHYSICAL EXAMINATION Her temperature on admission was 103.8° F. There were signs of lung involvement in the right middle lobe and lower part of the right upper lobe, which were confirmed by x rays.

COURSE. Cultures of the tooth sockets and sputum showed a mixture of mouth organisms with *Streptococcus viridans* predominating. Blood cultures

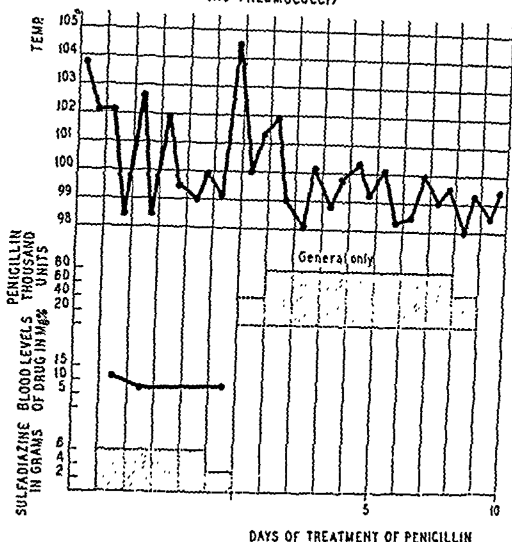


FIG. 3. There seemed to be little doubt that penicillin prevented the development of a lung abscess in this case (Courtesy of Dr William Herrick)

were negative. She was given sulfadiazine, 5 gm. for an initial dose and 1 gm. every 4 hours. The blood level promptly rose to 12 mg. per cent. Her temperature gradually came down to 100° F. on the fifth day although the x rays showed a spread of the pulmonary processes. On the sixth day she had another chill and her temperature rose to 104.6°. A lung abscess was suspected. Blood cultures were again negative. The sputum showed no pneumococci, but other mouth organisms with *Streptococcus viridans* predominating. On the sixth day, penicillin was started in at the rate of 10,000 units every 3 hours. The injections were exceedingly painful, but she accepted the treatment for a full course of 8 days. On the second day after treatment was begun, *she felt definitely better*

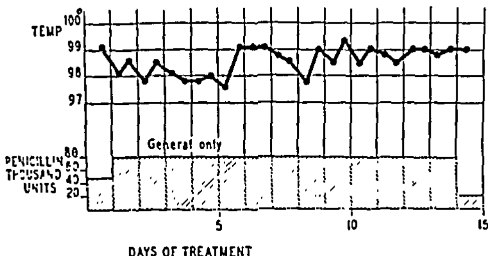


FIG. 4. A metastatic acute osteomyelitis which subsided with penicillin without the necessity for surgery (Courtesy of Dr. John Scudder)

although x rays revealed a spread to the left side with a clearing on the right. Three days later, both areas had cleared (Fig. 3)

This case illustrates embolic pneumonia with incipient lung abscess following extraction of teeth, responding satisfactorily to penicillin after sulfadiazine had failed.

Case IV: S.J. Age 18. No. 717648.

DIAGNOSIS. Acute hematogenous osteomyelitis of the radius.

HISTORY. Six years previously, this boy had developed a chronic hematogenous osteomyelitis of the tibia. Finally a radical excision of the diseased bone was done, the wound was packed, and the leg put up in plaster. Seven weeks after discharge from the hospital, he woke one morning to find his left forearm swollen and very painful above the wrist.

PHYSICAL EXAMINATION. His temperature on admission was 99.2° F. The left forearm, just above the wrist, was greatly swollen, red, hot, and tender. X rays revealed an abscess in the radius above the wrist. Before beginning treatment, 5 out of 7 doctors on the service gave their opinion that surgery would be required.

COURSE. He was started on 10,000 units of penicillin every 3 hours intramuscularly. There was no obvious improvement in 24 hours, but after 48 hours the pain began to subside, and the swelling began to recede rapidly. The symptoms steadily cleared up On the seventh day, however, x rays revealed further bone destruction, but in the absence of symptoms and signs, he left the hospital on the fifteenth day and returned to work 3 weeks later. Subsequent x rays showed a progressive regeneration of the bone. (Fig. 4)

This case illustrates the resolution of an acute osteomyelitis without the necessity for surgery, and the reconstruction of the sterilized necrotic bone.

Case V: T.R. Age 20. No. 730481.

DIAGNOSIS. Gangrenous ulcer of the dorsal surface of the left index finger with suppurative arthritis of the first interphalangeal joint.

HISTORY. Six weeks before admission a gangrenous infection developed at the side of the nail of the left index finger following a prick with steel wool. It slowly progressed, and 2 weeks before admission it spread to the first knuckle with cellulitis of the hand. On the day before admission, the overlying extensor tendon dissolved, exposing the joint. The author had never before seen such rapid dissolution of tendon tissue.

COURSE. The patient was given sulfadiazine, and although the temperature fell, the local process became worse. Penicillin was started both locally and generally. The temperature did not rise again and the wound almost immediately cleared up. On the fourth day of penicillin treatment, amputation was performed, leaving a long anterior flap. All of the cartilage had been digested from the head of the proximal phalanx. The wound was left open and treated with local penicillin, 5000 units daily. On the second day after operation the wound looked clean, and on the third day the flap was brought up and sutured in place. Local penicillin was applied between the sutures for 3 days and general penicillin continued in a dosage of 10,000 units every 3 hours. The wound healed by primary union. (Fig. 5.)

This case illustrates a severe gangrenous infection caused by a combination of hemolytic streptococcus and *Staphylococcus aureus* resulting in a dissolution of the extensor tendon and destruction of the first interphalangeal joint, treated with local and general penicillin. Amputation was done with a late primary closure. The wound promptly healed by primary union.

Case VI: H.W. Age 63, No. 741652.

DIAGNOSIS. Cellulitis of distal phalanx of thumb. Lymphangitis of forearm, hemolytic streptococcus septicemia.

HISTORY. On the day before admission, the patient developed a red, painful, swollen ball of the left thumb, which became progressively worse. He had had two attacks of coronary occlusion with cardiac failure during the previous 18 months, the last attack 2 days before admission There was marked swelling of the distal phalanx of the thumb with induration but no fluctuation There were red streaks running up the forearm toward the axilla.

COURSE. On the third day after his hospital admission, an incision was made in the distal anterior closed space of the thumb, but only cellulitis was found Cultures revealed a hemolytic streptococcus Next day there was lymphangitis and on the second day his temperature shot up to 100.2° F. A blood culture was taken that yielded the hemolytic streptococcus. He was put on penicillin 10,000 units every 3 hours. The temperature fell rapidly and the blood culture

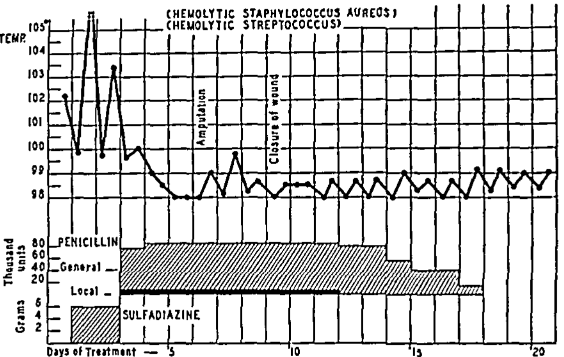


FIG. 5. A severe infection with a mixture of *organisms* which destroyed the cartilage of the joint was brought under control with penicillin and this permitted a late primary closure of the wound.

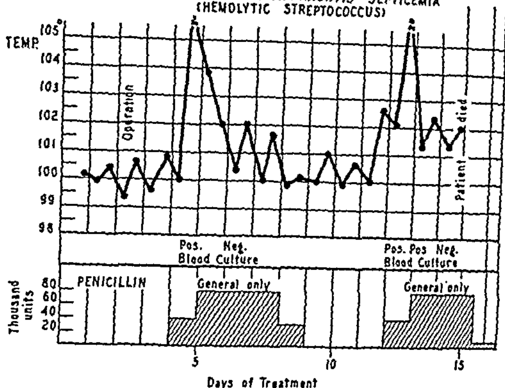


FIG. 6. *A repeated favorable response to penicillin. The recurrence of the infection was probably due to too early cessation of treatment. (Courtesy of Dr Harold Harvey)*

became sterile. After 4 days the penicillin was stopped, and the temperature rose again to 105 8° F. The blood became positive again. Fluid collected in both chests and the heart failed. Penicillin was started again in the same dosage and both chests were tapped. The chest fluid showed no growth on culture. The blood again became sterile, but venous pressure gradually mounted to 202°, and the patient died. (Fig. 6.)

This case illustrates the clearing of a positive blood culture from hemolytic streptococcus with penicillin and a recurrence after penicillin had been stopped. Readministration again cleared the picture, but the patient died of cardiac failure. The penicillin should have been continued in this case for a longer period of time, although we cannot be sure that it would have prevented a return of the septicemia, or the death from cardiac failure. This was an early case, treated when penicillin was very scarce.

Case VII: A.N. Age 65. No. 721021.

DIAGNOSIS. Abscesses of liver and acute diffuse peritonitis due to hemolytic *Staphylococcus aureus*.

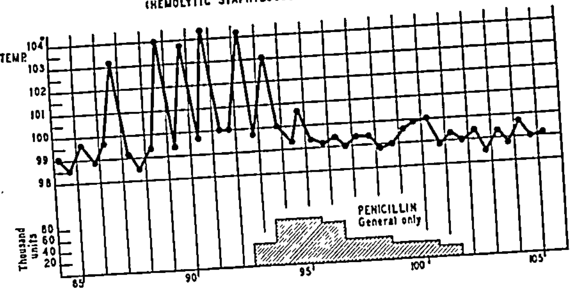


FIG. 7. An unusual case of multiple staphylococcal liver abscesses which responded strikingly to penicillin. (Courtesy of Dr. Harold Harvey)

HISTORY. The patient had had intermittent chills and fever for two months before admission. He later developed upper abdominal pain with almost daily chills. For the previous month he had been jaundiced.

PHYSICAL EXAMINATION. The patient appeared acutely and chronically ill, with rapid, feeble pulse. The abdomen was soft with no spasm, but there was tenderness over the liver.

COURSE. He had a chill on the second and third day after admission, temperature ranging up to 105.8°. On the third day, he was operated upon and pus was found throughout the upper abdomen with irregular areas on the undersurface of the liver, which were taken to be multiple abscesses. After the operation, he was given 6 gm. of sulfadiazine, and this was continued for 2 days. His temperature fell to normal, but spiked up to 101° on each of these days. On the third day sulfadiazine was discontinued, but his temperature began to mount again with recurrent chills and on the sixth day sulfadiazine was renewed with a second fall of temperature. It then ran within normal limits for the next 9 days, and sulfadiazine was again discontinued. On the twenty-fifth day following an operation, he again developed a chill, and this recurred on 4 of the next 5 days. Penicillin was then started at noon of the thirty-first postoperative day. The fever rose to 103° that evening but without a chill, and returned to normal the next morning, where it remained until discharge on the forty-third day. Penicillin was discontinued after the ninth day. (Fig. 7.)

This case illustrates a *Staphylococcus aureus* peritonitis with multiple liver abscesses recurring after two periods of improvement with sulfadiazine. After 6 days of spiking temperature with chills, penicillin was instituted and completely controlled the infection.

Case VIII: W.C. Age 16. No. 726260.

DIAGNOSIS. Cellulitis of leg and abscess of thigh due to a hemolytic *Staphylococcus aureus* and a nonhemolytic streptococcus.

HISTORY. Six days before admission a small furuncle developed just above the left knee joint. Next day, the infection spread, and the patient was put to bed with elevation and hot packs and given 1 gm. of sulfadiazine every 4 hours. On the fourth day his temperature mounted to 101.4° F. and the swelling and redness increased with a small amount of seropurulent discharge from the central point.

PHYSICAL EXAMINATION. On admission, his temperature was 101.8° F. There was a large area of cellulitis involving the outer and anterior surface of the thigh and extending on down to the leg with a small central discharging sinus.

COURSE. A blood culture was taken, which later showed no growth. He was put on penicillin every 3 hours intramuscularly and hot applications were applied. The temperature promptly fell to normal within 48 hours and remained there. The local signs steadily subsided until, on the sixth day, there were none. (Fig. 8.)

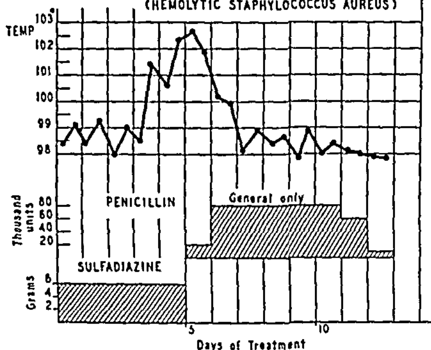


FIG 8. A favorable response to penicillin obviating surgery after sulfadiazine had failed to control the infection (Courtesy of Dr David Bull)

This case illustrates the rapid resolution of an alarming cellulitis in the region of the knee joint with very little localization of the infection threatening both septicemia and suppurative arthritis. It failed to respond to sulfadiazine and yielded promptly to penicillin. In this case there was a mixture of susceptible organisms that responded promptly to penicillin.

Case IX: E.S. Age 62. No. 631003.

DIAGNOSIS. Abscess of chest wall due to a hemolytic streptococcus.

HISTORY. Five weeks before admission, the patient ran a splinter into the web space between her left thumb and forefinger. A small abscess developed, which was drained. One week later, she developed pain and swelling in the left axilla. The patient treated it at home with heat lamp without improvement. The swelling increased and spread beneath the breast.

PHYSICAL EXAMINATION. On admission, the patient appeared acutely ill and in considerable discomfort. Her temperature was 103 4° F. She was found to have a huge 'water bottle' type of abscess occupying the entire left subpectoral and axillary areas. Although the lesion was acutely tender, there was practically no local heat or redness

COURSE. On the day following admission, 400 cc of thick, light green pus were aspirated from the abscess cavity. The exudate cultured a hemolytic

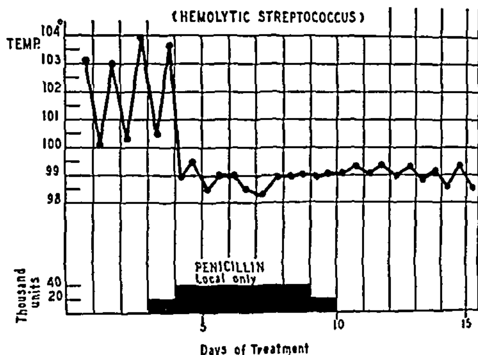


Fig. 9. A striking response to a limited form of surgery aided by the local application of penicillin

streptococcus. On the fourth day, a small incision was made at the lowest margin of the abscess, and 1000 cc. of the same type of exudate were released. Four Carrel tubes were inserted into the abscess cavity and 10,000 units of penicillin in 100 cc. of saline were instilled every 6 hours. The temperature promptly fell to normal, where it remained during the rest of her hospital stay. No general penicillin was given, but the local instillation of 40,000 units daily was continued for 6 days. (Fig. 9.)

This case illustrates the rapid resolution of a very large abscess of the axilla due to a hemolytic streptococcus from local instillation of penicillin with a limited surgical procedure and without any necessity for systemic treatment.

Case X: G.B. Age 27. Doctor's Hospital. (Private patient of my associate, Dr. H. D. Harvey.)

diagnosis Postpartum pelvic thrombophlebitis due to hemolytic *Staphylococcus aureus* and a nonhemolytic streptococcus.

history. The patient was delivered of a normal baby by means of forceps and episiotomy, following a difficult labor.

course. On the second day postpartum she had a chill with fever mounting to 103.6°. This spiking temperature recurred in the subsequent 5 days although not always accompanied by a chill. No benefit was obtained from sulfadiazine

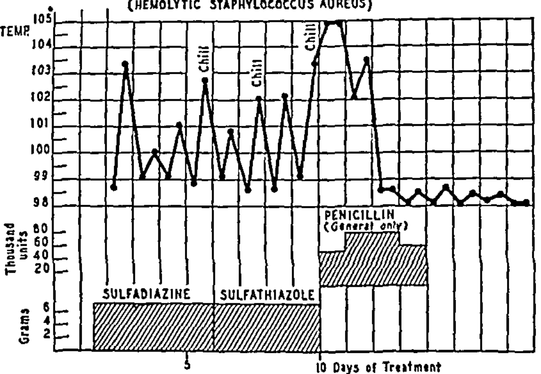


FIG. 10. A brilliant cure of a postpartum thrombophlebitis after both sulfadiazine and sulfathiazole had failed to control the infection. (Courtesy of Dr. Harold Harvey)

or sulfathiazole. The patient was acutely ill, with tenderness over the lower abdomen. Purulent lochia yielded on culture, *Staphylococcus aureus* and a nonhemolytic streptococcus. The penicillin was administered 10,000 units every 3 hours intramuscularly. The temperature fell to normal within 48 hours and remained there. The penicillin was continued for only 4 days. (Fig. 10.)

This case illustrates the prompt response to penicillin of a postpartum thrombophlebitis due to a mixture of susceptible organisms after failure with sulfadiazine and sulfathiazole. Although this patient was given penicillin for only 2 days after the temperature had fallen to normal and it was adequate for this case, it would have been wiser to have continued the treatment a little longer. This was an early case, treated when the supply of penicillin was limited.

Case XI: W.D. Age 10. No. 572074.

DIAGNOSIS. Acute suppurative arthritis of the knee and abscess of the external condyle of the femur due to hemolytic *Staphylococcus aureus*

HISTORY. Four and a half years before, a small sequestrum had been removed from a sinus tract leading down to the external condyle. The tract was then treated with staphylococcus bacteriophage and it promptly healed. There were no symptoms until a month before admission, when the patient had begun to have pain in the knee joint and in the lower part of the thigh. This had steadily increased until he was no longer able to walk.

PHYSICAL EXAMINATION. On examination, there was marked swelling and tenderness of the outer side of the femur above the knee joint. A large cavity occupied the external condyle of the femur and there was fluid in the knee joint.

COURSE The joint was aspirated, and a seropurulent fluid was obtained that revealed Gram-positive cocci on smear and that cultured hemolytic *Staphylococcus aureus*. Five thousand units of penicillin were introduced into the knee joint. The next day there was practically no fluid in the joint, so it was not opened However, the bony cavity was uncovered and the surface of the cavity was gently cleared of necrotic bone by means of a gouge. The cavity was then filled with penicillin and gently packed with a silk tampon and gauze. Each day 5000 units of penicillin were instilled into the cavity and the packing renewed. On the ninth day, the silk tampon was removed when the bone cavity was found to be lined with bright red granulations. They quickly filled the cavity, and the patient left the hospital on the twenty-seventh day. (Fig. 11.)

This case illustrates the prompt resolution of suppurative arthritis in the knee joint following a single aspiration and instillation of penicillin with complete return of function of the joint and the rapid healing of the bone cavity after removing the diseased bone, followed by the daily instillation of penicillin. No systemic drug was needed.

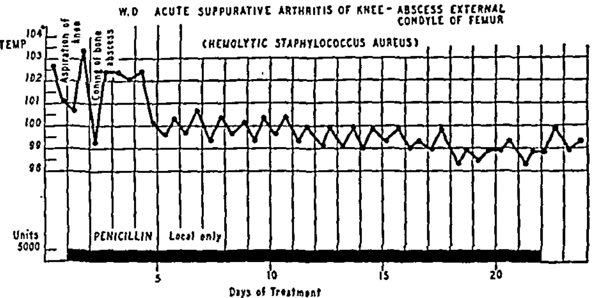


FIG. 11. A staphylococcal suppurative arthritis of the knee joint which was cured by a *single injection* of penicillin while a bone abscess of the *outer condyle* of the femur was cured by uncapping the abscess and applying penicillin locally

Case XII: R.M. Age 15. No. 494927.

DIAGNOSIS. Acute osteomyelitis of the ilium with *septicemia due to* hemolytic *Staphylococcus aureus.*

HISTORY. About 10 days before admission, a boil developed on the inner surface of the knee. While playing baseball, the boy bruised his hip, and that evening was thrown into a lake by his playmates. That night he developed a chill and a high fever and was sent to the hospital. A blood culture was taken that yielded hemolytic *Staphylococcus aureus.* He was given sulfathiazole and sulfadiazine without improvement and after a week's time was sent to the Presbyterian Hospital in New York.

PHYSICAL EXAMINATION. The boy was acutely ill with a swelling in the right buttock and tenderness over the right ilium. The hip joint was free. He was put on sulfadiazine without improvement, and on the second day an incision was made over the swelling on the right buttock, and an abscess was found deep to the gluteal muscles. At the same time he was given staphylococcus bacteriophage, but he showed no improvement and the organism was found to be resistant to the phage. Blood cultures again revealed hemolytic *Staphylococcus aureus.* He was then given penicillin at the rate of 10,000 units every 3 hours, and his temperature promptly fell. The penicillin was reduced to 5000 units every 3 hours, and discontinued after 6 days when his temperature reached normal. However, the temperature rose again and he began to show signs of pulmonary involvement. Penicillin was renewed, but again discontinued after 3 days. With the stopping of penicillin, the lung signs returned but again responded to penicillin therapy. With the complete subsidence of clinical symptoms he was allowed to go to a convalescent home (Fig. 12). He returned however, after 3 weeks, with fever and a tender mass in the right lower quadrant, which was thought to be an abscess developing on the inner surface of the ilium. The author believed that it would be necessary to incise this mass, but penicillin was tried first, at the rate of 5000 units every 3 hours. The temperature promptly fell and the mass rapidly resolved. The temperature did not rise again. He went again for convalescence and had no further recurrence of symptoms. X rays showed a progressive regeneration of the bone. (Fig. 13.)

This case was one of the first to be treated with penicillin at the Presbyterian Hospital. It illustrates the possibility of controlling septicemia with osteomyelitis and pulmonary involvement with relatively small doses of penicillin. The supply at that time was limited; otherwise the dosage would have been larger and the treatment continued longer. The case also shows that progressive degeneration of the bone may go on with the absence of clinical symptoms and that a recurrence of activity within the bone may respond to further treatment with penicillin without the necessity for surgery.

Case XIII. L.S. Age 30. No. 575675.

DIAGNOSIS Clostridial cellulitis (*C. sordellii*) of the arm.

HISTORY. Two days before admission to the hospital, because of an asthmatic attack, the patient had received a hypodermic injection of adrenalin, morphine,

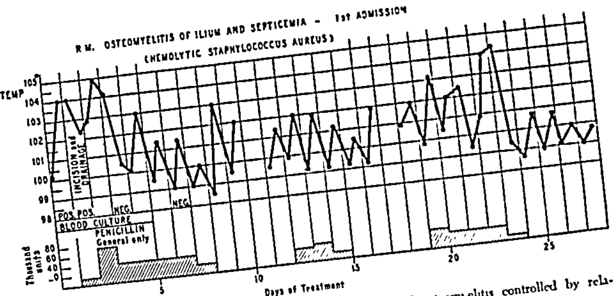


FIG. 12 One of the early cases of staphylococcal septicemia and osteomyelitis controlled by relatively small doses of penicillin.

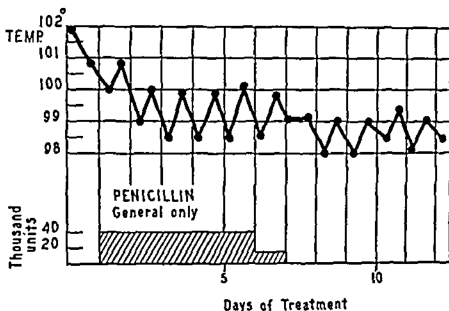


FIG. 13 A recurrence of activity in the bone focus promptly subsided with the reinstitution of penicillin treatment. Cure was effected without a surgical procedure on the bone

and atropine in the back of the left arm. The following day she was aware of swelling and tenderness in the region of the injection. These symptoms increased, and she became feverish. Shortly before admission to the hospital, she noted rapid increase of the rate of swelling and experienced periods of great prostration.

PHYSICAL EXAMINATION. The patient was admitted at 8:30 P.M., appearing acutely ill. Rectal temperature was 102.6° F. There was great swelling of the left upper extremity from the upper deltoid region almost to the elbow. The swelling extended around the upper arm and also involved the axilla, so that it had to be held away from the body. It was so tense that it suggested to the first doctor who saw her the possible need for releasing incisions through the deep fascia. There was some tenderness in the swollen area, most marked on the medial and axillary aspects. The lack of redness and heat was striking. There was no fluctuation. The radial pulse was difficult to feel at either wrist because her blood pressure remained low, at times being almost unobtainable.

COURSE. Treatment the first night consisted of high elevation of the arm, poultices to the upper arm, sulfadiazine, 1 gm. every 4 hours, with sodium bicarbonate, 0.5 gm. At times the patient was irrational. Next morning the swelling of the arm had extended throughout the forearm to the wrist. The blood pressure was hardly obtainable, although the patient was clear mentally. The medical consultant thought that the swelling could not be explained upon an allergic basis. X ray of the arm showed no gas in the tissues. There were

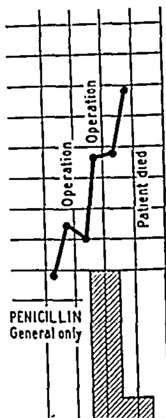


Fig 14a. An anaerobic cellulitis due to a mixture of *C. sordellii* and *C. welchii* not checked by penicillin. (Courtesy of Dr. John Scudder)

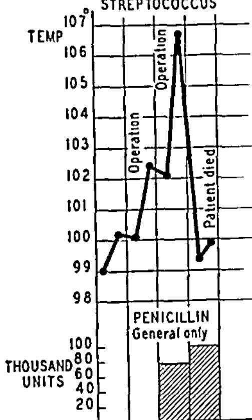


Fig. 14b. A case of gas gangrene of the abdominal wall possibly benefited but not cured by either penicillin or zinc peroxide (Courtesy of Dr. Harold Harvey)

still no certain signs of infection. The sulfadiazine was stopped after she had received 3 gm. in 12 hours.

OPERATION. An incision 10 cm. in length under local novocain anesthesia was made in the posterior aspect of the arm, where the hypodermic injection had been given, which was the probable source of the infection The subcutaneous tissues were extremely firm and grayish yellow in color Clear watery fluid exuded when the tissues were divided. There was practically no bleeding. The patient went into shock just before the operation. The tissues were divided through the brachialis fascia, and no pus or gas was encountered. The underlying muscle appeared normal. The wound was tamponed open with China silk. About the time of operation 800 cc. of pooled plasma and 700 cc. of saline

solution were given by infusion. The swelling of the arm increased in spite of profuse discharge of serous fluid from the incision. A hematocrit taken shortly before midnight was 57.8; plasma specific gravity, 1.0217; plasma protein, 5.03. The blood in the tube was hemolyzed. Soon after midnight further multiple incisions through the deep fascia were made into the area of swelling, because of the extreme tension of the tissues. The blood pressure was at this time unobtainable. Pulse was about 100. 500 cc. of whole blood were given. A generalized urticaria followed. Pathological examination of a specimen of the subcutaneous tissue was later reported to show scattered small foci of lymphoid infiltration, but nothing suggestive of acute inflammation. Cultures next day from swabs moistened in the wound revealed *Clostridium sordellii* and *C. welchii*. On the basis of this finding, 50,000 units of sodium penicillin were given intramuscularly, and during the next 14 hours 200,000 units were given intravenously. Thereafter, 10,000 units were given every 3 hours intramuscularly, until her death, 22 hours after the first dose of penicillin, for a total of 280,000 units. There was no evidence of any beneficial effect from this treatment. (Fig. 14a.)

Postmortem examination revealed the following significant abnormal findings: swelling and edema of the subcutaneous tissues of the left upper extremity with extension into the left side of the thorax and the neck. In one region of the left pectoralis muscle were many areas of necrosis and hemorrhage. There were 200 cc. of clear yellow fluid in the peritoneal cavity, 250 cc. in the right pleural cavity, and 750 cc. in the left, with partial atelectasis of both lower lobes. Microscopic sections of the subcutaneous fat of the left arm showed edema, many small hemorrhages, perivascular accumulations of polymorphonuclear leucocytes, which were undergoing degeneration, and a pale homogeneous coagulum within the walls and the lumina of some of the vessels. Gram-positive and Gram-negative rods were observed in one area. *Clostridium sordellii* and *Clostridium welchii* were grown from the operative wounds.

This case illustrates an overwhelming case of anaerobic cellulitis due primarily to *C. sordellii*, which showed no benefit from penicillin therapy or from surgical incisions.

Case XIV: E.H. Age 50. No. 588936.

DIAGNOSIS. Postoperative gas gangrene of the abdominal wall.

HISTORY. The patient had had repeated attacks of pain in the right upper quadrant, associated on occasions with chills, fever, and jaundice.

PHYSICAL EXAMINATION. Essentially negative, except for jaundice.

COURSE. There were no abnormal laboratory findings. A cholecystogram revealed no evidence of a gall-bladder shadow. She was operated on with a diagnosis of chronic cholecystitis and cholelithiasis with stone in the common duct. A right subcostal incision was made, and a calculus was removed from the duct. A probe could be readily passed into the duodenum and into both hepatic ducts. The ducts were thoroughly irrigated with saline solution. The bile in the common duct was muddy, and was subsequently shown to contain *C. welchii*, *Escherichia coli*, and *Streptococcus viridans*. There was no evidence

of an acute inflammatory process. Exploration of the abdomen revealed no other abnormality. The appendix was removed, a T tube was fastened into the common duct, a soft rubber drain placed alongside it, and the wound was closed about the drains in layers, by means of interrupted silk sutures.

For 20 hours the postoperative course was not thought to be unusual. During the morning of the first postoperative day, however, it was noticed that the patient was lethargic, that there was a good deal of serous discharge from the wound, and that bloody drainage was coming from the rubber tube, although bile was coming from the T tube. There was also found to be a hard, painful, tender area above the wound extending up toward the breasts. The pulse by noon had risen to 130 and temperature to 103.6° F. When the wound was dressed, the amount of discharge was noted to be excessive, but the odor was not thought to suggest anaerobic infection There was an area extending up from the wound that was swollen, hard, tender, and quite sharply outlined, corresponding roughly to the extent of the upper portion of the right rectus muscle. The lower margin of the wound was not affected The wound was opened, but it was not until some sutures were taken out of the anterior sheath exposing the muscle that a little gas was seen bubbling up. A smear taken of the exposed muscle showed Gram-positive rods that looked like *C. welchii*, and later were proved to be. (*Aerobacter aerogenes* and a nonhemolytic streptococcus were also cultured from the wound.) An x ray of the wound area taken on the way to the operating room did not reveal gas in the tissues.

The second operation was done about 33 hours after the first, and consisted of removal of the portion of the rectus muscle cephalad to the original wound. It was almost completely destroyed by gas gangrene, but no other area of invasion was recognized. The wound was packed wide open with zinc peroxide. Before operation, 50,000 units of penicillin were given intravenously, and 25,000 units at 3-hour intervals until the patient died, a total of 175,000 units in 16 hours. Starting about 3 hours after the second operation, she received 330 cc. of polyvalent gas-gangrene serum intravenously. Her temperature during the night fell dramatically from 106.6°, shortly before midnight, to 99.4 at 8.00 A.M. It did not again rise. Her pulse meanwhile fell from 130 before operation to 100 the next day, where it remained almost until she died. Nevertheless, her blood pressure fell, breathing became wheezy, and she began to cough after the second injection of antitoxin. For that reason, only half the dose was given. Subsequent doses of antitoxin did not have this effect, but breathing remained somewhat difficult and moist, and she became jaundiced. In spite of the drop in temperature and pulse, she looked more ill. She died at 3:40 P.M., 52 hours after the first operation. (Fig. 14b.)

At autopsy, virtually no evidence of active infection was found. No *C. welchii* were grown from cultures of the wound. The patient did have edema of the glottis and some edema of the lungs, and also a rheumatic heart with a rather tight mitral stenosis. The jaundice was unexplained. It was not due to persisting anaerobic infection, but may have been the result of the period of toxemia. The presence of bile plugs in the canaliculi suggested a

factor of obstruction, but no cause of obstruction was found. The liver was not apparently the site of an infection.

The case is shown to illustrate a fatal outcome in a case of postoperative gas gangrene in which penicillin was of questionable value. It would appear that the gas-gangrene infection had been controlled, which might have been the result of any or all of the therapeutic measures taken, that is, the surgical removal of the focus, the zinc peroxide, the penicillin, and the serum. The patient's death might have been caused by any one of a number of factors, such as the period of toxemia, too much intravenous fluid (although the hematocrit remained within normal limits), failure of a rheumatic heart, serum sensitivity, or edema of the glottis. Death was probably due not directly to gas gangrene, but indirectly to it and to the chain of events that accompanied it.

Case XV: S.W. Age 4. No. 593762.

DIAGNOSIS. Anaerobic cellulitis and myositis of arm, shoulder girdle, and chest.

HISTORY Four days before admission the boy was knocked down by a school bus and his left forearm was caught under the wheel, a lacerated wound of the extensor muscles being produced. He was taken at once to the local hospital where the wound was débrided, but not all of the damaged tissue and foreign bodies were removed. Sulfathiazole powder was generously applied to the wound surfaces, which were loosely closed with interrupted stitches. Two days later, the forearm and hand began to swell and edema rapidly spread upward toward the shoulder and axilla and under the chest wall. The process was not in any way halted by sulfadiazine. On the fourth day the edema had advanced across the chest and back to the opposite shoulder and a diagnosis of an anaerobic cellulitis was made. He was transferred to Presbyterian Hospital and he was immediately operated on.

OPERATION Extensive edema was found in the subcutaneous tissue over the right pectoral muscle, gradually increasing in thickness (as was seen when the exploratory incisions were made) over the left pectoral muscle and *in the axilla and over the anterior surface of the biceps* muscles. Gram-positive rods were found in the serous exudate from all of these wounds. The wound itself was then explored and the extensor muscles were found to be the site of a foul-smelling gangrene. The gangrenous process was found to extend upward and involve the triceps and brachialis anticus muscles all the way up to the shoulder joint. This condition necessitated amputation, which was done in the upper quarter of the humerus, the necrotic muscles being excised right up to their origin. There was little or no gas in the tissues but there was an extensive edema, the cultures yielding a great mixture of Gram-positive, spore-forming anaerobes including *C. welchii*, *C septicum*, and *C. tetani*, as well as hemolytic *Staphylococcus aureus* and nonhemolytic streptococcus.

COURSE He was given 100,000 units of penicillin 2 hours before operation and 50,000 units after operation every 3 hours for 6½ days, the local wound

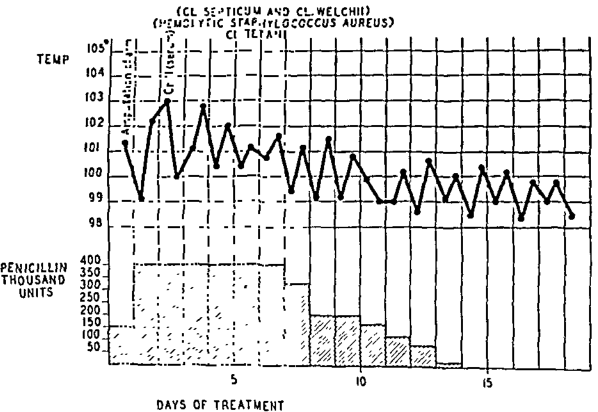


Fig. 15. An extensive anaerobic cellulitis and myositis controlled by penicillin and anti-gas gangrene serum after the necrotic muscles had been removed by amputation

being treated with zinc peroxide suspended in distilled water. He was also given 13 therapeutic doses of Lederle's polyvalent gas-gangrene serum over a period of 36 hours. The progress of the edema promptly stopped and it gradually receded. Granulations developed rapidly in the wound and the infection came completely under control. On the eighth day the dose of penicillin was cut in half and continued at the rate of 25,000 units every 3 hours for the next 3 days. Then for 2 days the dose was reduced to 15,000 and for 1 day to 5000 units, for a total of 3,565,000 units over a period of 14 days. (Fig. 15.)

This case illustrates the development of anaerobic cellulitis in an accidental wound in which local sulfathiazole and general sulfadiazine *failed to prevent the infection* when the wound was incompletely débrided and loosely closed. Infection spread rapidly as a cellulitis with extensive edema but minimal gas formation. Necrosis of the principal muscles of the arm required amputation. Although all the grossly gangrenous muscle was removed by surgical procedure and the surface of the wound was treated with zinc peroxide there was a large area of cellulitis that could only be reached by the penicillin and gas-gangrene serum, both of which probably played a role in the patient's recovery.

Case XVI: M.R. Age 30. No. 838901.

DIAGNOSIS. Osteomyelitis of the os pubis.

HISTORY. Eleven months before admission, the patient noticed a pain in the right inguinal region over the right pubic bone. A swelling gradually developed which reached a diameter of about 5 cm. in the course of two months. It was then opened and pus was released. The wound did not heal but continued to drain pus in small quantities up to the time of admission. Lipiodol was injected into the sinus and an x-ray film was taken, which revealed a sinus tract leading down into a cavity in the os pubis (see Fig. 16). Culture revealed a coagulase positive hemolytic *Staphylococcus aureus*. This organism was relatively resistant to penicillin but was susceptible to a concentration of 100 units per cc.

OPERATION. The tract was excised and the abscess cavity in the os pubis was coned out smoothly. A tampon of China silk was soaked with penicillin in a concentration of 5000 units per cc. and laid on the surface of the bone cavity. The tampon was then packed with gauze wet with penicillin in the same concentration and the wound was covered with vaseline gauze.

COURSE. No systemic penicillin was used but the packing was removed and replaced daily each time instilling 5000 units of penicillin in 1 cc. into the cavity. Granulations grew up rapidly lining the bone cavity and gradually filling the wound. On the 19th day, penicillin in a carbowax (water soluble) base in a concentration of 500 units per gram was applied to the granulating wound. Healing progressed rapidly and the patient left the hospital on the 26th day with the wound healed. A subsequent x ray showed regeneration of the bone (see Fig. 17).

This case illustrated the treatment of a chronic staphylococcus abscess of the bone by excision and local application of concentrated penicillin



FIG. 16 An osteomyelitic cavity in the right os pubis with a tract down to it outlined with lipiodol

solution with a prompt control of the infection, a rapid development of granulation tissue and complete healing of the wound with regeneration of the bone.

Case XVII: S.W. Age 14. No. 731135 (Private patient of my confrere, Dr. Beverly Smith.)

DIAGNOSIS. Hematogenous osteomyelitis of the femur. Diabetes mellitus.

HISTORY. This young girl had been treated for severe diabetes since the age of three. Ten weeks before admission, she fell down and struck her knee. Two days later she developed a blister on her heel from a tight shoe. Three days later she began to have pain in her left knee with general malaise and fever. She was taken to a local hospital, where a blood culture revealed a hemolytic *Staphylococcus aureus*. There was no improvement following the administration of sulfadiazine. Penicillin was obtained from Doctor Chester Keefer and she was given 275,000 units over a period of 5 days. However, this did not control the septicemia, and the blood culture remained positive for 5 weeks. At that time more penicillin was obtained and given in a dosage of 133,000 units a day for 9 days. By this treatment, the septicemia was finally controlled, but repeated x-ray films of the femur revealed progressive necrosis. She was then transferred to the Presbyterian Hospital in New York.

PHYSICAL EXAMINATION. The patient was an emaciated, pale, seriously ill girl with marked swelling of the lower two thirds of the thigh and the region of the knee joint. An x-ray film of the left femur is shown in Figure 18. It reveals an extensive destruction of the lower half of the femur with involve-



FIG. 17. The tract and bone cavity have been excised and the resulting wound packed with a silk tampon and gauze wet with penicillin. Only local drug was used once daily, first in solution form and then in a carbowax ointment base. The cavity quickly filled in and the wound healed.

ment of both condyles and virtual sequestration of the lower third of the bone The red blood count was 3,700,000. The white cells were 14,850 with 76 per cent of polys. The temperature was only a 100° but mounted to 101° on the second day.

COURSE After consultation with many doctors, it was the consensus of opinion that any operative procedure short of amputation would almost certainly result in a fracture of the femur with dire consequences. At that time the supply of penicillin was meager but it was deemed justified to apply it to this case for a few days to see if it would have any effect on halting the process of destruction and permit the development of an involucrum which would permit the trauma of a sequestrectomy. Penicillin was therefore administered in a dosage of 20,000 units every 3 hours and transfusions were given at frequent intervals. There was definite improvement in the patient's general condition and the local signs of inflammation slowly began to subside, but after 2 weeks of treatment improvement came to a standstill. The supply of penicillin was running low and at that time it seemed advisable to proceed with amputation unless a large supply of penicillin could be obtained to continue treatment for at least a month. This was granted following a special appeal to Doctor Keefer, and treatment was continued. At the beginning of the eighth week of hospitalization, a thick involucrum had formed, as shown in Figure 19. The patient's general condition was fair; the diabetes was under control; so an operation was performed. This removed the sequestrum of the shaft but did not disturb the epiphesis. The cavity was packed with a



FIG. 20. The main focus in the shaft has been excised and the cavity filled with a silk tampon and gauze packing soaked with penicillin. Foci still remain in the epiphesis.



FIG 21. The infection has now been removed and the wound is healed. The bone is showing reconstruction.

similarly treated (Fig. 20). Granulations rapidly grew up and all wounds were finally healed 19 weeks after admission. During her stay in the hospital, she received a total of 11,365,000 units of penicillin systemically and 410,000 units locally. The bone healing is shown in Figure 21.

This case illustrates one of the early triumphs of penicillin and reveals the control of a staphylococcus septicemia with a relatively small dose

of penicillin, but the establishment of a bone focus with progressive destruction and sequestration requiring surgical intervention. However, with continued systemic administration of penicillin as well as local application of it, the infection finally came under control and the limb as well as the life of the patient was saved. The case also illustrates the possibility of control of secondary contaminants, such as pyocyaneus and proteus by the local application of parachlorophenol.

F. Results in a Series of 744 Established Surgical Infections

The foregoing records bring out certain features of penicillin treatment as exemplified by individual cases. In order to obtain a better understanding of what might be expected from penicillin treatment in the general run of surgical infections, the Subcommittee on Surgical Infections and Burns of the National Research Council undertook a study of established surgical infections in the several units that had been set up to study the prevention of infection in civilian accidental wounds simulating war wounds. The data from 744 cases were gathered together with the aid of summary sheets and were carefully analyzed. The results were classified in four categories as 'excellent,' 'good,' 'questionable,' and 'no effect.' These were published in the *Annals of Surgery* in 1946.[22]

The excellent results were clearly manifest within 72 hours, the good within a week or 10 days, the others were doubtful or obviously negative. Taking the group as a whole, about 15 per cent yielded 'excellent' results, 50 per cent were 'good,' while the rest were equally divided between 'questionable' and 'no effect.' The cases fell naturally into three groups: (1) most favorable, with 86 per cent to 92 per cent of satisfactory results; (2) intermediate, with 50 per cent to 75 per cent favorable responses; and (3) least favorable, with less than 50 per cent responding to the treatment. In the first group, in the order of their favorable results, were found furuncles, cellulitis, mastoiditis, carbuncles, suppurative arthritis, lung abscesses, superficial abscesses, brain abscesses, and osteomyelitis.

In the second group were deep abscesses, thrombophlebitis, sinusitis, infected soft-part wounds, infected operative wounds, otitis media, infected compound fractures, and ulcers of the skin. In the third group were found empyemas, infected burns, gas gangrene, actinomycosis, and miscellaneous infections, with postoperative pneumonia, peritoneal abscesses, and diffuse peritonitis at the very bottom of the list.

The series could be divided into three groups according to the method of treatment, 438 were given systemic treatment only, 164 had local and general administration, and 142 received local application only, in the form of solution or ointment. These three groups are not com-

parable, but it is important to note that a large number of cases yielded promptly to local treatment only, with very economical expenditure of the drug.

Significant facts were brought out by the bacteriologic studies in these cases. The majority of the infections yielded on culture a mixture of organisms. The hemolytic streptococcus was all alone in only 16 per cent of the cases in which it was found, the coagulase positive staphylococci, in about 35 per cent. The pathogenic Gram-negative rods in only 5 per cent, and the Welch bacillus in only 3 per cent. Results were regularly better in cases with a pure culture rather than a mixture of organisms except with the Gram-negative rod infections where penicillin had little effect. The highest percentage of favorable results were found in the pure staphylococcal infections, reaching almost 90 per cent.

In an analysis of the 131 cases in which penicillin had no effect, there were found 58 cases in which organisms were present that had the power of producing penicillinase. Other circumstances militating against a favorable outcome were an associated tuberculosis, diabetes, tetanus, or resistant staphylococci or streptococci, or too small dosage, too late treatment, or too conservative surgery.

Among the 82 cases in the series having septicemia, the best results were obtained in the hemolytic streptococcus group, with 87.5 per cent recoveries. The staphylococcus group yielded 69 per cent recoveries, a fatal outcome being usually explained by an endocarditis with widespread metastases often established before treatment had begun.

As a result of this study, four points warrant special emphasis:

1. Penicillin is a very valuable adjunct to surgery in the treatment of surgical infections and in many instances it can obviate surgery, but it is not a panacea.

2. Penicillin is effective when applied locally to well-localized surgical infections if the organisms are susceptible and not antagonistic.

3. 'Adequate dosage' is the amount that will bring the infection under control and get the patient well, and it cannot be determined in any given case before the beginning of treatment.

4. A careful and complete bacteriologic analysis is essential to obtain the best results.

II. OTHER ANTIBIOTICS

A. STREPTOMYCIN

The failure of penicillin to control infections due to Gram-negative rods stimulated those who were searching throughout the antibiotic field for potent agents, to further efforts directed toward the control of these organisms.

Waksman finally discovered among the large group of actinomyces

an organism that had the power of producing a relatively nontoxic antibiotic, which he has called Streptomycin.[23, 24] Difficulties were encountered in commercial manufacture similar to those met with in the production of penicillin. It was particularly difficult to eliminate some of the toxic products that developed during the process of manufacture. For a time it was necessary to maintain complete control of the output in order to make it available to the Armed Forces, and during that period it was possible to make observations only in military hospitals. Finally in the summer of 1946, it became available for suitable civilian cases.

For its appraisal in army hospitals, a control unit was set up under the direction of Captain Edwin J. Pulaski at the Halloran Hospital in New York City. The cases were studied carefully before, during, and after the administration of this new agent. This study included the identification of the causative organisms and a test of their sensitivity to Streptomycin. Blood studies, urinalysis, renal-function tests, spinal-fluid examinations, and roentgenograms were made in all cases.

The case records were collected in a uniform manner with the aid of summary sheets covering the essential features of the cases. Similar summary sheets were used for gathering data in other military hospitals, which however did not have such complete laboratory controls. The data submitted from all units were studied and analyzed by Colonel DeBakey and Captain Pulaski who made a report of their findings, which may be summarized as follows: [25]

Of the 706 cases analyzed, slightly more than half were urinary infections. Three-fifths of these urinary infections occurred in patients with spinal cord injuries and less than a third of these were benefited. In patients without cord injury, approximately 60 per cent were benefited. The favorable results occurred chiefly in those urinary tract infections that were due to a pure culture of a susceptible organism without any urinary calculi, local cellulitis, or abscess. The organisms most uniformly susceptible were those of the aerobacter, coli, and Friedländer groups.

The next largest group of cases reported by these authors were chronically infected wounds. These represented about a quarter of the total number. All these wounds were incurred in combat, and most of them were associated with infection of the bone or neighboring soft tissues. The flora of these wounds was mixed, staphylococci, streptococci, clostridia, and Gram-negative bacteria of fecal origin usually playing important roles. Forty per cent of these cases were considered to be improved by the administration of Streptomycin, while the rest were definitely not benefited. In the favorable cases, however, improvement was usually coincident with adequate wound revision. The authors believed that the surgery was more important than the drug although

they had to concede that the drug played a definitely favorable role in the improvement or in the *recovery from the infection* in two-fifths of the cases.

The results in peritonitis were favorable in 80 per cent of the cases although they state that the results were not 'dramatic,' suggesting that other factors may have been partly or largely responsible for the recoveries. This medication was effective in colitis when the *specific organisms* were found to be susceptible to the drug, but non-specific ulcerative colitis did not respond favorably.

These investigators found that with inadequate doses, organisms often became resistant to the drug, and they advocated large doses of 300 to 500 mg. every 4 hours to avoid this. Favorable results were obtained in *Hemophilus influenzae* meningitis following intrathecal administration. Tularemia responded in two instances. Cases of gonorrheal urethritis that had previously resisted the sulfonamides and penicillin were promptly cured by Streptomycin.

According to these studies, Streptomycin has a definite place in the treatment of surgical infections particularly as an adjunct to surgery and in the complication of infections of the urinary tract that so frequently follow surgical procedures.

B. BACITRACIN

Another relatively non-toxic antibiotic that has been made available for the treatment of surgical infections is Bacitracin. This is produced by a Gram-positive sporulating bacillus of the *B. subtilis* group that was isolated from a bacterial mixture found in the débrided tissue from a compound fracture cultured in our own laboratory. The early laboratory product seemed to be entirely void of toxicity, but in the early commercial preparations certain toxic elements appeared that have delayed the systemic administration of the antibiotic to human beings. However, with the relatively small quantities required for the local treatment of surgical infections, observations were made and recorded by the author on 100 human cases,[26] while laboratory observations were being carried on in our own bacteriological research laboratory in the department of surgery and in the chemical and pharmacological laboratories of the College of Physicians and Surgeons.[27-9]

The analysis of 100 cases of surgical infections demonstrated that favorable results might be expected in 85 per cent to 90 per cent of the ordinary run of surgical infections coming to a surgeon's office or to a surgical clinic. In 31 per cent of these cases the results were classified as excellent with a prompt response and rapid resolution within 72 hours of the initiation of treatment. In 57 per cent the response was unmis-

takable but slower—the resolution generally coming within a week or ten days. The diagnosis and the results obtained are shown in the accompanying table.

TABLE I

RESULTS OBTAINED IN THE FIRST HUNDRED CASES OF SURGICAL INFECTIONS TREATED BY THE LOCAL APPLICATION OF BACITRACIN

DIAGNOSIS	RESULTS OF TREATMENT				
	Total Cases	Excellent	Good	Questionable	No Effect
Furuncle (boil)	16	8	8	0	0
Deep abscess	13	4	9	0	0
Superficial abscess	10	3	7	0	0
Infected sebaceous cyst	9	0	8	1	0
Infected operative wound	9	4	5	0	0
Multiple furuncles	6	0	4	2	0
Carbuncle	4	1	3	0	0
Undermining ulcer	4	2	4	0	0
Subungual abscess	4	2	2	0	0
Chronic osteomyelitis	3	0	1	1	1
Ulcer of old scar	3	0	2	1	0
Stye	3	3	0	0	0
Infected accidental wound	2	1	0	1	0
Ulcer of leg	2	0	1	1	0
Ulcer of vulva	2	0	1	1	0
Impetigo	2	2	0	0	0
Miscellaneous (1 each)	8	3	2	1	2
Totals	100	31	57	9	3

Favorable 88%.

In 62 of these cases, no surgery was performed other than aspiration. In 18, a surgical incision would almost certainly have been required before the advent of antibiotics. Among these were several anterior closed space infections of the hands and both superficial and deep abscesses of the face above the 'danger line.'

The bacteriological studies in these cases revealed the fact that the majority (52.3 per cent) were due to a mixture of organisms. Only the coagulase positive staphylococcus appeared more often in pure than in mixed cultures. The Gram-negative bacilli were always associated with other organisms. Their frequent presence in surgical infections and their ability to produce penicillinase is probably the chief single cause of the

failure of penicillin in the treatment of mixed infections. The fact that they produce no inactivation of Bacitracin suggests that the latter may be *more effective than penicillin in the* treatment of these polymicrobial lesions.

When the cocci found in this series were tested for susceptibility to both penicillin and bacitracin, it was found that 30 yielded to bacitracin but not to penicillin, while only 6 were resistant to bactracin and susceptible to penicillin.

The systemic administration of bacitracin is still in its initial stages and its appraisal will have to await the careful study of a large series of cases representing all kinds of infectious diseases.

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Numbers in italics indicate references in bibliography.

INDEX

INDEX